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TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: FCCC.99-08US

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INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US00/40789	31 August 2000	20 September 1999

TITLE OF INVENTION: NUCLEIC ACID ENCODING HUMAN ABCA TRANSPORTER 2 (ABCA2) AND METHODS OF USE THEREOF

APPLICANT(S) FOR DO/EO/US: TEW, Kenneth D.; VULEVIC, Bojana; CHEN, Zhijian

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [X] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [ ] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [ ] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [ ] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment.
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
18. [ ] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [X] Other items or information:
    Copy of Form PCT/IB/308

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U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/088467 not yet assigned	PCT/US00/40789	FCCC.99-08US

21. [X] The following fees are submitted:

	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 100.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	25 - 20 =	5	x **$18.00**	$ 90.00	
Independent claims	8 - 3 =	5	x **$84.00**	$ 420.00	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$280.00**	$ 0	
TOTAL OF ABOVE CALCULATIONS =				$ 610.00	
[ ] Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.			+	$ 305.00	
SUBTOTAL =				$ 305.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				$ 0	
TOTAL NATIONAL FEE =				$ 305.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **$40.00** per property +				$ 0	
TOTAL FEES ENCLOSED =				$ 305.00	
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a. [X] A check in the amount of $ 305.00 to cover the above fees is enclosed

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NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

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43,047
REGISTRATION NUMBER

Nucleic Acid Encoding Human ABCA Transporter 2 (ABCA2) and Methods of Use Thereof

By Kenneth D. Tew
Bojana Vulevic
Zhijian Chen

Pursuant to 35 U.S.C. §202(c) it is acknowledged that the U.S. Government has certain rights in the invention described herein, which was made in part with funds from the National Institutes of Health, Grant Number, 5 R35 CA53893-07.

FIELD OF THE INVENTION

The present invention relates to the fields of medicine and molecular biology. More specifically, the invention provides nucleic acid molecules and proteins encoded thereby which are involved in the regulated transport of biological and pharmacological molecules across cell membranes.

BACKGROUND OF THE INVENTION

Several publications are referenced in this application in parentheses in order to more fully describe the state of the art to which this invention pertains. The disclosure of each of these publications is incorporated by reference herein.

The ABC transporters are members of the family of cellular membrane proteins responsible for unidirectional movement of many different substrates across biological membranes in prokaryotes and eukaryotes (Higgins, 1992). These ABC transporters share structural similarity with most having one or two highly conserved ATP binding cassette (ABC) regions and one or two highly hydrophobic domains often with six transmembrane segments (Luciani et al, 1994). Commonly, family members have a two plus two structure and are regarded as full-size transporters, as opposed to half transporters which contain one ATP

binding cassette and one hydrophobic domain. ABC transporters localize to plasma membranes (P-glycoprotein, MRPs) or to the internal membranes of different organelles (ALD, TAP, ABC7, M-ABC1). Many ABC transporters play important roles in the cellular efflux of endogenous or xenobiotic substrates, and their biological dysfunction has been implicated in a number of clinical disorders. For example, impaired function of the cAMP activated CFTR chloride channel appears to be the basic defect in epithelial and non-epithelial cells derived from cystic fibrosis patients (Kunzelmann, 1999). Mutations in peroxisomal membrane half-transporters, ALDP and PMP70 are associated with abnormal peroxisomal beta-oxidation of saturated, very long chain fatty acids giving rise to neurodegenerative disorders such as X-linked adrenoleukodistrophy and Zellweger syndrome (Smith et al., 1999; Collins and Could, 1999). Aberrant expression of half-transporters, such as TAP-transporters (de la Salle et al., 1994; Cucca et al., 1994) has been linked to diseases like bare lymphocyte syndrome I and insulin-dependent diabetes mellitus. These proteins localize to endoplasmic reticulum and are involved in antigen processing. The mitochondrial half-transporter, ABC7 has been associated with a range of hereditary diseases in humans (Savary et al., 1997; Shimada et al., 1998; Csere et al., 1998; Allikmets et al., 1999). The mutations in this iron-transporter are responsible for X-linked sideroblastic anemia and ataxia (XLSA/A). More recently, ABCR or ABCA4, the rod photoreceptor ABC transporter has been implicated in a whole spectrum of vision disorders (Evans and Bhattacharya, 1998; Rozet et al., 1998; Shroyer et al., 1999; Maugeri et al., 1999; Lewis et al., 1999). Mutations in the ABCR gene interfere with transport functions which can lead to conditions associated with

Stargard Disease, AMD (age-related macular degeneration), Fundus Flavimaculatus, Cone-Rod Dystrophy and Retinitis Pigmentosa. Kinetic analysis of the ATPase activity of ABCR (Sun et al., 1999) and characterization of abcr knockout mice (Weng et al., 1999) showed that ABCR probably functions as an outwardly directed flippase for N-retinylidene-phosphatidylethanolamine, protecting the retinal pigment epithelium from toxic adducts of photobleaching. ABC1, a mammalian homologue of the C. elegans ced-7 gene, has been shown to be required for phagocytosis of both necrotic and apoptotic cells (Luciani and Chimini, 1996; Mounaylt et al., 1998;) and is involved in macrophage interleukin-1 secretion (Hamon et al., 1997). This protein has also been found to function as a cholesterol pump and some mutations in the ABC1 gene are causative for Tangier disease and familial high-density cholesterol deficiency with high predisposition for atherosclerosis (Langmann et al., 1999; Brooks-Wilson et al., 1999; Bodzioch et al., 1999; Rust et al., 1999.) The mechanism(s) underlying these apparently loosely related functions has yet to be addressed.

Extending the drug resistance paradigm, research from our laboratory on the ABCA2 transporter revealed that amplification of the *ABCA2* gene was linked to resistance to estramustine (Laing et al., 1998). Both gene copy number and mRNA levels of this transporter were increased in the resistant cell line. In addition, antisense treatment directed toward *ABCA2* mRNA sensitized the resistant cells to estramustine. Together, these results suggested that ABCA2 is causally involved in estramustine resistance and implied a possible role for ABCA2 in steroid transport.

SUMMARY OF THE INVENTION

This invention provides novel, biological molecules useful for identification, detection, and/or molecular characterization of components involved in the transport of molecules across cell membranes. According to one aspect of the invention, an isolated nucleic acid molecule is provided which includes a sequence encoding a full length human ABCA2 protein transporter of a size about 2436 amino acids in length. The encoded protein, referred to herein as human ABCA2 comprises a multi-domain structure including a tandem repeat of nucleotide binding folds appended to a hydrophobic domain that contains several potential membrane spanning helices. Conserved Walker A and B ATP binding sites are present in each of the nucleotide binding folds.

In a preferred embodiment of the invention, an isolated nucleic acid molecule is provided that includes a cDNA encoding a human ABCA2 protein. In a particularly preferred embodiment, the human ABCA2 protein has an amino acid sequence the same as Sequence I.D. No. 2. An exemplary human *ABCA2* nucleic acid molecule of the invention comprises Sequence I.D. No. 1.

According to another aspect of the present invention, an isolated nucleic acid molecule is provided, which has a sequence selected from the group consisting of: (1) Sequence I.D. No. 1; (2) a sequence specifically hybridizing with preselected portions or all of the complementary strand of Sequence I.D. No. 1 comprising nucleic acids encoding amino acids 1-20, 1-35, 1-40, 1-60 and 1-150 of Sequence ID No. 2; (3) a sequence encoding preselected portions of Sequence I.D. No. 1 within nucleotides 1-200.

Such partial sequences are useful as probes to identify and isolate homologues of the human *ABCA2* genes of the invention. Additionally, isolated nucleic acid

sequences encoding natural allelic variants of the nucleic acids of Sequence I.D. No. 1 are also contemplated to be within the scope of the present invention. The term natural allelic variants will be defined hereinbelow.

According to another aspect of the present invention, antibodies immunologically specific for the human ABCA2 proteins described hereinabove are provided.

In yet another aspect of the invention, host cells comprising the human *ABCA2* encoding nucleic acid of the invention are provided. Such host cells include but are not limited to bacterial cells, fungal cells, insect cells, mammalian cells, and plant cells. Host cells overexpressing the human *ABCA2* encoding nucleic acids of the invention provide valuable research tools for assessing transport of chemotherapeutic agents out of cells. Human ABCA2 expressing cells also comprise a biological system useful in methods for identifying inhibitors of the ABCA2 transporters.

Another embodiment of the present invention encompasses methods for screening cells expressing the human ABCA2 encoding nucleic acids for chemotherapy resistance. Such methods will provide the clinician with data which correlates expression of a particular human *ABCA2* gene with a particular chemotherapy resistant phenotype.

Diagnostic methods are also contemplated in the present invention. Accordingly, suitable oligonucleotide probes are provided which hybridize to the nucleic acids of the invention. Such probes may be used to advantage in screening biopsy samples for the expression of mutated *ABCA2* genes.

The methods of the invention may be applied to kits. An exemplary kit of the invention comprises *ABCA2* gene specific oligonucleotide probes and/or primers, *ABCA2*

encoding DNA molecules for use as a positive control, buffers, and an instruction sheet. A kit for practicing the cell line screening method includes frozen cells comprising the human *ABCA2* encoding nucleic acid of the invention, suitable culture media, buffers and an instruction sheet.

In a further aspect of the invention, transgenic knockout mice are disclosed. Mice will be generated in which the *ABCA2* gene has been knocked out. Such mice will provide a valuable biological system for assessing resistance to chemotherapy in an in vivo tumor model.

Various terms relating to the biological molecules of the present invention are used hereinabove and also throughout the specification and claims. The terms "percent similarity" and "percent identity (identical)" are used as set forth in the UW GCG Sequence Analysis program (Devereux et al. NAR 12:387-397 (1984)).

With reference to nucleic acids of the invention, the term "isolated nucleic acid" is sometimes used. This term, when applied to DNA, refers to a DNA molecule that is separated from sequences with which it is immediately contiguous (in the 5' and 3' directions) in the naturally occurring genome of the organism from which it originates. For example, the "isolated nucleic acid" may comprise a DNA or cDNA molecule inserted into a vector, such as a plasmid or virus vector, or integrated into the genomic DNA of a prokaryote or eukaryote.

With respect to RNA molecules of the invention, the term "isolated nucleic acid" primarily refers to an RNA molecule encoded by an isolated DNA molecule as defined above. Alternatively, the term may refer to an RNA molecule that has been sufficiently separated from RNA molecules with which it would be associated in its natural state (i.e., in cells or tissues), such that it exists in a "substantially pure" form (the term

"substantially pure" is defined below).

With respect to protein, the term "isolated protein" or "isolated and purified protein" is sometimes used herein. This term refers primarily to a protein produced by expression of an isolated nucleic acid molecule of the invention. Alternatively, this term may refer to a protein which has been sufficiently separated from other proteins with which it would naturally be associated, so as to exist in "substantially pure" form.

The term "substantially pure" refers to a preparation comprising at least 50-60% by weight the compound of interest (e.g., nucleic acid, oligonucleotide, protein, etc.). More preferably, the preparation comprises at least 75% by weight, and most preferably 90-99% by weight, the compound of interest. Purity is measured by methods appropriate for the compound of interest (e.g. chromatographic methods, agarose or polyacrylamide gel electrophoresis, HPLC analysis, and the like).

With respect to antibodies of the invention, the term "immunologically specific" refers to antibodies that bind to one or more epitopes of a protein of interest (e.g., human ABCA2), but which do not substantially recognize and bind other molecules in a sample containing a mixed population of antigenic biological molecules.

With respect to nucleic acids and oligonucleotides, the term "specifically hybridizing" refers to the association between two single-stranded nucleotide molecules of sufficiently complementary sequence to permit such hybridization under pre-determined conditions generally used in the art (sometimes termed "substantially complementary"). When used in reference to a double stranded nucleic acid, this term is intended to signify that the double stranded nucleic acid has been subjected to denaturing conditions, as is well known to

those of skill in the art. In particular, the term refers to hybridization of an oligonucleotide with a substantially complementary sequence contained within a single-stranded DNA or RNA molecule of the invention, to the substantial exclusion of hybridization of the oligonucleotide with single-stranded nucleic acids of non-complementary sequence.

One common formula for calculating the stringency conditions required to achieve hybridization between nucleic acid molecules of a specified sequence homology (Sambrook et al., 1989):

T_m = 81.5°C + 16.6Log [Na+] + 0.41(% G+C) - 0.63 (% formamide) - 600/#bp in duplex

As an illustration of the above formula, using [Na+] = [0.368] and 50% formamide, with GC content of 42% and an average probe size of 200 bases, the T_m is 57°C. The T_m of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C. Such sequences would be considered substantially homologous to the nucleic acid sequences of the invention.

The nucleic acids, proteins, antibodies, cell lines, methods, and kits of the present invention may be used to advantage to identify targets for the development of novel agents which inhibit the aberrant transport of biological and pharmacological agents into and out of cells. The transgenic mice of the invention may be used in an in vivo model for chemotherapy resistance.

The human ABCA2 molecules, methods and kits described above may also be used as research tools and will facilitate the elucidation of the mechanism by which cellular transport may be augmented or inhibited.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1. Diagram showing overlapping clones used to define the full-length cDNA of ABCA2. The white boxes represent the untranslated regions. A 1.75 kb fragment was originally PCR amplified. Clones designated as λ represent clones isolated from oligo(dT) and random primed human fetal brain phage library. Other clones were isolated using RACE PCR with human brain Marathon cDNA. The only exception is clone AB028985, which stands for GenBank accession number of brain KIAA1062 protein. The most 5' clones, 65 and 119 contain the ATG start codon designated at position 1 representing a previously described sequence of a brain cDNA (GenBank Accession Number AB028985).

Figs. 2A, 2B, 2C and 2D. Primary structure analysis of human ABCA2. Fig. 2A: Amino acid sequence predicted from the full length *ABCA2* cDNA. The 12 predicted *transmembrane-spanning segments* are indicated with a heavy bar below the sequence and the corresponding numerals below. The 21 predicted N-glycosylation sites on the extracellular surface are indicated by "*". Residues comprising the extended ATP-binding cassettes are underlined and indicated by "ATP binding cassette". The highly hydrophobic domain is underlined and labeled as "HHD". The nucleotide sequence was deposited in GenBank under accession number AF 178941. Fig. 2B: Kyle-Doolittle hydrophobicity analysis of ABCA2 protein. The shaded areas above line are hydrophobic and those below are hydrophilic. Analysis was performed in GCG software. Fig. 2C: Schematic showing the predicted topology of ABCA2 in the membrane. The extracellular surface is on the top and the cytoplasmic on the bottom of the figure. The stylized rods on the cytoplasmic loop show N-glycosylation sites. Fig. 2D: Dot-matrix plot of human

ABCA2 against itself, with PAM 250 matrix and a window of 8 residues, shows little sequence similarity outside the nucleotide folds.

Fig. 3. Multialignment of the amino acid sequences of the extended nucleotide binding cassettes from the group of ABC1-similar proteins. Clustal-W analysis was performed in Mac Vector software. Shading highlights the conserved residues. Underlined are ATP binding cassette sequence motifs: WA stands for Walker motif A; WB stands for Walker motif B and ATS corresponds to active transport signature. The reported sequences were extracted from the GenBank database, under the following Accession numbers: ABCA4 (human): NM_000341; ABCA3(human): NM_001089; ABCA2(human) AF178941; ABCA1(human): AAF86276; abca2 (mouse): CAA53531.

Fig. 4. Multiple Northern analysis blot. Blot containing 1 μg of polyA+ RNA from each tissue hybridized with an 870 bp *ABCA2* probe (top panel) or with a β-actin probe (bottom panel).

Fig. 5. Tissue distribution of the human *ABCA2*. A human MTE Array (Clontech, Palo Alto, CA) was probed with a 840 bp biotin labeled fragment of *ABCA2*. Hybridization, detection and quantitation of signal are described further hereinbelow. A1-10: whole brain, cerebral cortex, frontal lobe, parietal lobe, occipital lobe, temporal lobe, cerebral cortex, pons, cerebellum-left, cerebellum-right; B1-10: corpus callosum, amygdala, caudate nucleus, hippocampus, medulla oblongata, putamen, substantia nigra, accumbens nucleus, thalamus, pituitary gland; C1-10: spinal cord, heart, aorta, atrium-left, atrium-right, ventricle-left, ventricle-right, interventricular septum, apex of the

heart, esophagus; D1-10: stomach, duodenum, jejunum, ileum, ilocecum, appendix, colon-ascending, colon-transverse, colon-descending, rectum; E1-10: kidney, skeletal muscle, spleen, thymus, peripheral blood leukocyte, lymph node, bone marrow, trachea, lung, placenta; F1-10: bladder, uterus, prostate, testis, ovary, liver, pancreas, adrenal gland, thyroid gland, salivary gland; G1-10: mammary gland, leukemia HL-60, HeLa S3, leukemia K-562, leukemia MOLT-4, Burkitt's lymphoma-Raji, colorectal adeno-carcinoma-SW480, lung carcinoma-A549, fetal brain; H1-10: fetal heart, fetal kidney, fetal liver, fetal spleen, fetal thymus, fetal lung, yeast total RNA, yeast tRNA, E.coli rRNA, E. coli DNA; I1-10:Poly r(A), human Cot_1 DNA, human DNA 100 ng, human DNA 500 ng.

Figs. 6A and 6B. Sequence ID No. 1.

Fig. 7. Sequence ID No. 2.

DETAILED DESCRIPTION OF THE INVENTION

An isolated human *ABCA2* encoding nucleic acid, the protein encoded thereby and antibodies immunologically specific for the ABCA2 protein are provided in the present invention. Based on the degree of amino acid identity ABCA2 can be regarded as an ortholog of mouse *ABCA2* (Luciani et al., 1994). This protein is closely related to members of ABC1-subfamily of transporters, ABCA1, ABCA4 and ABCA3 and more distantly related to MDR1 (Chen et al., 1986), MRP1(Riordan et al., 1989) and CFTR (Zielenski et al., 1991).

The topology of ABCA2 is reminiscent of that observed in ABCA4 (Illing et al., 1997; Azarian and Travis, 1997; Rozet at al., 1998; Nasonkin et al., 1998). The large size of this protein (2436 amino acids and

calculated MW ~ 270 kDa) makes this protein one of the largest ABC transporters reported to date. The extracellular loop between the first two transmembrane segments together with the regulatory domain account for the size difference compared to other full-size transporters.

ABCA2 has a large number of possible sites for both glycosylation and phosphorylation. A total number of 21 putative glycosylation sites, confirms the glycolytic character of the protein and suggests that functional regulation may be accomodated by phosphokinases. The activity of a related abc transporter, ABCA1 is upregulated *in vitro* upon treatment with protein kinase A (Becq et al.,1997), implying a possible similar role in ABCA2.

The distribution of *ABCA2* shows prevalence of expression in the central nervous system, brain and spinal cord. This is in agreement with the data of Luciani and colleagues who showed similar tissue distribution of mouse *abca2* (Luciani et al., 1994). Lower expression is observed in lymphoid tissue, such as appendix and spleen together with thyroid gland. So far, several ABC transporters have been identified in the blood-brain barrier (e.g. p-glycoprotein, MRP1) and their role in the transport of xenobiotics is well characterized. ABCA2 may have a similar role in brain. However, although its prevalence in neuronal tissue supports an important role for ABCA2 in this tissue, its presence in other tissues suggests a more pleiotropic role for this transporter. This concept is further supported by the broad expression pattern in the tumor cell lines examined. High ABCA2 levels may be linked to the transformed phenotype or to the physical characteristics of growing cells *in vitro*. Expression may also be influenced by maturation and/or hormone changes

since transcript levels in mouse uterus increase significantly during pregnancy (Luciani et al.,) and in adult compared to fetal brain.

In a number of cases, ABC transporters have been shown to participate in active transport of a variety of drugs, lipids, metabolites and peptides (Endicott and Ling, 1989; Hettema et al., 1996; Ewart et al., 1994; Berkow and Michaelis, 1991; Powis et al., 1992). The data from our earlier study on estramustine-resistance clearly implicate ABCA2 in resistance to this drug in an ovarian carcinoma cell line (Laing et al., 1998). Estramustine is a synthetic nitrogen mustard derivative of estradiol with an unexpected antimitotic activity. Thus although it cannot be a natural substrate for ABCA2, it maintains a structural similarity to the steroid estradiol (Punzi et al., 1992). Another possible clue to function of ABCA2 is the presence of lipocalin signature motifs in the regulatory domain of protein. Lipocalins are a family of proteins linked to transport of retinoids, steroids (including cholesterol) lipids and bilins (Flower, 1996). They are characterized by the presence of an eight anti-parallel beta sheet peptide conformation (~ 200 amino acids long) that form a binding site for the hydrophobic substrates. Perhaps not coincidentally, the locus of some members of this family is on 9q34, were ABCA2 resides. In addition, ABCA2-similar proteins (ABCA1 and ABCA4) are involved in the transport of retinoids and cholesterol. Thus, it is likely that steroid, lipid or other similar substrates bind to this lipocalin component of ABCA2, facilitating their transport. Alternatively, the high CNS expression of the transporter may imply that substrates such as neurotransmitters, ions and/or bioacive peptides or amino acids are transported by ABCA2 protein.

I. Preparation of *ABCA2*-Encoding Nucleic Acid Molecules, ABCA2 Proteins, and Antibodies Thereto

A. Nucleic Acid Molecules

Nucleic acid molecules encoding the ABCA2 proteins of the invention may be prepared by two general methods: (1) synthesis from appropriate nucleotide triphosphates, or (2) isolation from biological sources. Both methods utilize protocols well known in the art. The availability of nucleotide sequence information, such as cDNAs having Sequence I.D. No. 1 enables preparation of an isolated nucleic acid molecule of the invention by oligonucleotide synthesis. Synthetic oligonucleotides may be prepared by the phosphoramidite method employed in the Applied Biosystems 38A DNA Synthesizer or similar devices. The resultant construct may be purified according to methods known in the art, such as high performance liquid chromatography (HPLC). Long, double-stranded polynucleotides, such as a DNA molecule of the present invention, must be synthesized in stages, due to the size limitations inherent in current oligonucleotide synthetic methods. Thus, for example, a 5 kb double-stranded molecule may be synthesized as several smaller segments of appropriate complementarity. Complementary segments thus produced may be annealed such that each segment possesses appropriate cohesive termini for attachment of an adjacent segment. Adjacent segments may be ligated by annealing cohesive termini in the presence of DNA ligase to construct an entire 5 kb double-stranded molecule. A synthetic DNA molecule so constructed may then be cloned and amplified in an appropriate vector.

Nucleic acid sequences encoding the ABCA2 proteins of the invention may be isolated from appropriate biological sources using methods known in the art. In a preferred embodiment, a cDNA clone is isolated from a

cDNA expression library of human origin. In an alternative embodiment, utilizing the sequence information provided by the cDNA sequence, human genomic clones encoding ABCA2 proteins may be isolated. Alternatively, cDNA or genomic clones having homology with *ABCA2* may be isolated from other species using oligonucleotide probes corresponding to predetermined sequences within the *ABCA2* encoding nucleic acids.

In accordance with the present invention, nucleic acids having the appropriate level of sequence homology with the protein coding region of Sequence I.D. Nos. 1 may be identified by using hybridization and washing conditions of appropriate stringency. For example, hybridizations may be performed, according to the method of Sambrook et al., (supra) using a hybridization solution comprising: 5X SSC, 5X Denhardt's reagent, 1.0% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA, 0.05% sodium pyrophosphate and up to 50% formamide. Hybridization is carried out at 37-42°C for at least six hours. Following hybridization, filters are washed as follows: (1) 5 minutes at room temperature in 2X SSC and 1% SDS; (2) 15 minutes at room temperature in 2X SSC and 0.1% SDS; (3) 30 minutes-1 hour at 37°C in 1X SSC and 1% SDS; (4) 2 hours at 42-65°in 1X SSC and 1% SDS, changing the solution every 30 minutes.

Nucleic acids of the present invention may be maintained as DNA in any convenient cloning vector. In a preferred embodiment, clones are maintained in a plasmid cloning/expression vector, such as pBluescript (Stratagene, La Jolla, CA), which is propagated in a suitable *E. coli* host cell.

ABCA2-encoding nucleic acid molecules of the invention include cDNA, genomic DNA, RNA, and fragments thereof which may be single- or double-stranded. Thus, this invention provides oligonucleotides (sense or

antisense strands of DNA or RNA) having sequences capable of hybridizing with at least one sequence of a nucleic acid molecule of the present invention, such as selected segments of the cDNA having Sequence I.D. No. 1. Such oligonucleotides are useful as probes for detecting or isolating *ABCA2* genes. Antisense nucleic acid molecules may be targeted to translation initiation sites and/or splice sites to inhibit the translation of the *ABCA2*-encoding nucleic acids of the invention. Such antisense molecules are typically between 15 and 30 nucleotides and length and often span the translational start site of *ABCA2* encoding mRNA molecules. Nucleic acid sequences encoding antisense molecules corresponding to amino acids 1-10, 1-45, 1-50, 1-100 and 1-500 of SEQ ID NO: 2 are thus contemplated to be within the scope of the present invention.

It will be appreciated by persons skilled in the art that variants of these sequences exist in the human population, and must be taken into account when designing and/or utilizing oligos of the invention. Accordingly, it is within the scope of the present invention to encompass such variants, with respect to the *ABCA2* sequences disclosed herein or the oligos targeted to specific locations on the respective genes or RNA transcripts. With respect to the inclusion of such variants, the term "natural allelic variants" is used herein to refer to various specific nucleotide sequences and variants thereof that would occur in a human population. The usage of different wobble codons and genetic polymorphisms which give rise to conservative or neutral amino acid substitutions in the encoded protein are examples of such variants. Additionally, the term "substantially complementary or homologous" refers to nucleic acid sequences that may not be perfectly matched to a target sequence, but the mismatches do not

materially affect the ability of the oligo to hybridize with its target sequence under the conditions described.

B. Proteins

Full-length ABCA2 proteins of the present invention may be prepared in a variety of ways, according to known methods. The proteins may be purified from appropriate sources, e.g., transformed bacterial or animal cultured cells or tissues, by immunoaffinity purification. However, this is not a preferred method due to the low amount of protein likely to be present in a given cell type at any time. The availability of nucleic acid molecules encoding ABCA2 proteins enables production of the proteins using *in vitro* expression methods known in the art. For example, a cDNA or gene may be cloned into an appropriate *in vitro* transcription vector, such as pSP64 or pSP65 for *in vitro* transcription, followed by cell-free translation in a suitable cell-free translation system, such as wheat germ or rabbit reticulocytes. *In vitro* transcription and translation systems are commercially available, e.g., from Promega Biotech, Madison, Wisconsin or Gibco-BRL, Gaithersburg, Maryland.

Alternatively, according to a preferred embodiment, larger quantities of ABCA2 proteins may be produced by expression in a suitable prokaryotic or eukaryotic system. For example, part or all of a DNA molecule, such as a cDNA having Sequence I.D. No. 1, may be inserted into a plasmid vector adapted for expression in a bacterial cell, such as *E. coli*. Such vectors comprise the regulatory elements necessary for expression of the DNA in the host cell positioned in such a manner as to permit expression of the DNA in the host cell. Such regulatory elements required for expression include promoter sequences, transcription initiation sequences and, optionally, enhancer sequences.

The human ABCA2 proteins produced by gene expression in a recombinant prokaryotic or eukaryotic system may be purified according to methods known in the art. In a preferred embodiment, a commercially available expression/secretion system can be used, whereby the recombinant protein is expressed and thereafter secreted from the host cell, to be easily purified from the surrounding medium. If expression/secretion vectors are not used, an alternative approach involves purifying the recombinant protein by affinity separation, such as by immunological interaction with antibodies that bind specifically to the recombinant protein or nickel columns for isolation of recombinant proteins tagged with 6-8 histidine residues at their N-terminus or C-terminus. Alternative tags may comprise the FLAG epitope or the hemagglutinin epitope. Such methods are commonly used by skilled practitioners.

The human ABCA2 proteins of the invention, prepared by the aforementioned methods, may be analyzed according to standard procedures. For example, such proteins may be subjected to amino acid sequence analysis, according to known methods.

As mentioned above, the human *ABCA2* encoding nucleic acid of the invention may include a sequence that differs slightly from the SEQ ID NO:1, yet encodes a polypeptide having the same amino acid sequence. Alternatively, the encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from those shown in SEQ ID NO: 2. Nucleic acid encoding a polypeptide which is an amino acid sequence mutant, variant or derivative of the sequence shown is further provided by the present invention. Nucleic acid encoding such a polypeptide may show at the nucleotide sequence and/or encoded amino acid level greater than about 60% homology with the relevant coding or encoded sequence

shown herein, greater than about 70% homology, greater than about 80% homology, greater than 90% homology and preferably greater than 95% homology to the sequences provided herein. For amino acid "homology", this may be understood to be similarity (according to established principles of amino acid similarity), e.g., as determined using the algorithm GAP (Genetics Computer Group, Madison, WI) or identity. GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, the default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of GAP may be preferred but other algorithms may be used, e.g., BLAST (which uses the method of Altschul et al, (1990) J. Mol. Biol. 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) PNAS USA 85:2444-2448), or the Smith-Waterman algorithm (Smith and Waterman (1981) J. Mol. Biol. 147:195-197), generally employing default parameters. Use of either of the terms "homology" and "homologous" herein does not imply any necessary evolutionary relationship between compared sequence in keeping with the meaning of the term as used in the phrase "homologous recombination". As is well known to those of skill in the art, homologous recombination merely requires that two nucleotide sequences be sufficiently similar to recombine under the appropriate conditions.

The present invention also provides antibodies capable of immunospecifically binding to proteins of the invention. Polyclonal antibodies directed toward human ABCA2 proteins may be prepared according to standard methods. In a preferred embodiment, monoclonal antibodies are prepared, which react immunospecifically with the various epitopes of the ABCA2 proteins described herein. Monoclonal antibodies may be prepared according

to general methods of Köhler and Milstein, following standard protocols. Polyclonal or monoclonal antibodies that immunospecifically interact with ABCA2 proteins can be utilized for identifying and purifying such proteins. For example, antibodies may be utilized for affinity separation of proteins with which they immunospecifically interact. Antibodies may also be used to immunoprecipitate proteins from a sample containing a mixture of proteins and other biological molecules. Other uses of anti-ABCA2 antibodies are described below.

II. Uses of *ABCA2*-Encoding Nucleic Acids, ABCA2 Proteins and Antibodies Thereto

Cellular transporter molecules have received a great deal of attention as potential targets of therapeutic agents designed to effectively block the export of, or promote import of pharmacological reagents across cell membranes. The ABCA2 proteins of the invention play a pivotal role in cellular transport.

Additionally, *ABCA2* nucleic acids, proteins and antibodies thereto, according to this invention, may be used as research tools to identify other proteins that are intimately involved in the transport of molecules into and out of cells. Biochemical elucidation of molecular mechanisms which govern such transport will facilitate the development of novel anti-transport agents that may, for example, sensitize tumor cells to conventional chemotherapeutic agents.

A. *ABCA2*-Encoding Nucleic Acids

ABCA2-encoding nucleic acids may be used for a variety of purposes in accordance with the present invention. *ABCA2*-encoding DNA, RNA, or fragments thereof may be used as probes to detect the presence of and/or expression of genes encoding ABCA2 proteins. Methods in

which *ABCA2*-encoding nucleic acids may be utilized as probes for such assays include, but are not limited to: (1) *in situ* hybridization; (2) Southern hybridization (3) northern hybridization; and (4) assorted amplification reactions such as polymerase chain reactions (PCR).

The *ABCA2*-encoding nucleic acids of the invention may also be utilized as probes to identify related genes from other animal species. As is well known in the art, hybridization stringencies may be adjusted to allow hybridization of nucleic acid probes with complementary sequences of varying degrees of homology. Thus, *ABCA2*-encoding nucleic acids may be used to advantage to identify and characterize other genes of varying degrees of relation to the *ABCA2* genes of the invention. Such information enables further characterization of transporter molecules which give rise to the chemoresistant phenotype of certain tumors. Additionally, they may be used to identify genes encoding proteins that interact with ABCA2 proteins (e.g., by the "interaction trap" technique), which should further accelerate identification of the components involved in transport of biological molecules across cell membranes. The *ABCA2*-encoding nucleic acids may also be used to generate primer sets suitable for PCR amplification of target *ABCA2* DNA. Criteria for selecting suitable primers are well known to those of ordinary skill in the art.

Nucleic acid molecules, or fragments thereof, encoding *ABCA2* genes may also be utilized to control the production of ABCA2 proteins, thereby regulating the amount of protein available to participate in biological or pharmacological reagent transport. As mentioned above, antisense oligonucleotides corresponding to essential processing sites in *ABCA2*-encoding mRNA molecules may be utilized to inhibit ABCA2 protein

production in targeted cells. Alterations in the physiological amount of ABCA2 proteins may dramatically affect the ability of these proteins to transport pharmacological reagents out of the cell.

Host cells comprising *ABCA2*-encoding DNA molecules are encompassed in the present invention. Host cells contemplated for use in the present invention include but are not limited to bacterial cells, fungal cells, insect cells, mammalian cells, and plant cells. Methods for introducing DNA molecules are also well known to those of ordinary skill in the art. Such methods are set forth in Ausubel et al. eds., Current Protocols in Molecular Biology, John Wiley & Sons, NY, NY 1995, the disclosure of which is incorporated by reference herein.

The availability of *ABCA2*-encoding nucleic acids enables the production of strains of laboratory mice carrying part or all of the ABCA2 gene or mutated sequences thereof. Such mice may provide an *in vivo* model for development of novel therapeutic agents. Alternatively, the *ABCA2* nucleic acid sequence information provided herein enables the production of knockout mice in which the endogenous gene encoding *ABCA2* has been specifically inactivated. Methods of introducing transgenes in laboratory mice are known to those of skill in the art. Three common methods include: 1. integration of retroviral vectors encoding the foreign gene of interest into an early embryo; 2. injection of DNA into the pronucleus of a newly fertilized egg; and 3. the incorporation of genetically manipulated embryonic stem cells into an early embryo.

The alterations to the *ABCA2* gene envisioned herein include modifications, deletions, and substitutions. Modifications and deletions render the naturally occurring gene nonfunctional, producing a "knock out" animal. Substitutions of the naturally occurring gene

for a gene from a second species results in an animal which produces an *ABCA2* gene from the second species. Substitution of the naturally occurring gene for a gene having a mutation results in an animal with a mutated ABCA2 protein. A transgenic mouse carrying the human *ABCA2* gene is generated by direct replacement of the mouse ABC2 gene with the human gene. These transgenic animals are valuable for use in in vivo assays for elucidation of other medical disorders associated with cellular activities modulated by the *ABCA2* gene. A transgenic animal carrying a "knock out" of an *ABCA2*-encoding nucleic acid is useful for the establishment of a nonhuman model for chemotherapy resistance involving ABCA2 regulation.

As a means to define the role that ABCA2 plays in mammalian systems, mice can be generated that cannot make ABCA2 proteins because of a targeted mutational disruption of the *ABCA2* gene.

The term "animal" is used herein to include all vertebrate animals, except humans. It also includes an individual animal in all stages of development, including embryonic and fetal stages. A "transgenic animal" is any animal containing one or more cells bearing genetic information altered or received, directly or indirectly, by deliberate genetic manipulation at the subcellular level, such as by targeted recombination or microinjection or infection with recombinant virus. The term "transgenic animal" is not meant to encompass classical cross-breeding or in vitro fertilization, but rather is meant to encompass animals in which one or more cells are altered by or receive a recombinant DNA molecule. This molecule may be specifically targeted to a defined genetic locus, be randomly integrated within a chromosome, or it may be extrachromosomally replicating DNA. The term "germ cell line transgenic animal" refers

to a transgenic animal in which the genetic alteration or genetic information was introduced into a germ line cell, thereby conferring the ability to transfer the genetic information to offspring. If such offspring in fact, possess some or all of that alteration or genetic information, then they, too, are transgenic animals.

The alteration or genetic information may be foreign to the species of animal to which the recipient belongs, or foreign only to the particular individual recipient, or may be genetic information already possessed by the recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene. The altered *ABCA2* gene generally should not fully encode the same ABCA2 protein native to the host animal and its expression product should be altered to a minor or great degree, or absent altogether. However, it is conceivable that a more modestly modified *ABCA2* gene will fall within the compass of the present invention if it is a specific alteration.

The DNA used for altering a target gene may be obtained by a wide variety of techniques that include, but are not limited to, isolation from genomic sources, preparation of cDNAs from isolated mRNA templates, direct synthesis, or a combination thereof.

A preferred type of target cell for transgene introduction is the embryonal stem cell (ES). ES cells may be obtained from pre-implantation embryos cultured in vitro. Transgenes can be efficiently introduced into the ES cells by standard techniques such as DNA transfection or by retrovirus-mediated transduction. The resultant transformed ES cells can thereafter be combined with blastocysts from a non-human animal. The introduced ES cells thereafter colonize the embryo and contribute to the germ line of the resulting chimeric animal.

One approach to the problem of determining the

contributions of individual genes and their expression products is to use an isolated *ABCA2*-encoding nucleic acid to selectively inactivate the wild-type gene in totipotent ES cells (such as those described above) and then generate transgenic mice. The use of gene-targeted ES cells in the generation of gene-targeted transgenic mice is known in the art.

Techniques are available to inactivate or alter any genetic region to a mutation desired by using targeted homologous recombination to insert specific changes into chromosomal alleles. However, in comparison with homologous extrachromosomal recombination, which occurs at a frequency approaching 100%, homologous plasmid-chromosome recombination was originally reported to only be detected at frequencies between 10^{-6} and 10^{-3}. Nonhomologous plasmid-chromosome interactions are more frequent occurring at levels 10^5-fold to 10^2-fold greater than comparable homologous insertion.

To overcome this low proportion of targeted recombination in murine ES cells, various strategies have been developed to detect or select rare homologous recombinants. One approach for detecting homologous alteration events uses the polymerase chain reaction (PCR) to screen pools of transformant cells for homologous insertion, followed by screening of individual clones. Alternatively, a positive genetic selection approach has been developed in which a marker gene is constructed which will only be active if homologous insertion occurs, allowing these recombinants to be selected directly. One of the most powerful approaches developed for selecting homologous recombinants is the positive-negative selection (PNS) method developed for genes for which no direct selection of the alteration exists. The PNS method is more efficient for targeting genes which are not expressed at high levels because the

marker gene has its own promoter. Non-homologous recombinants are selected against by using the Herpes Simplex virus thymidine kinase (HSV-TK) gene and selecting against its nonhomologous insertion with effective herpes drugs such as gancyclovir (GANC) or (1-(2-deoxy-2-fluoro-B-D arabinofluranosyl)-5-iodouracil, (FIAU). By this counter selection, the number of homologous recombinants in the surviving transformants can be increased.

As used herein, a "targeted gene" or "knock-out" is a DNA sequence introduced into the germline or a non-human animal by way of human intervention, including but not limited to, the methods described herein. The targeted genes of the invention include DNA sequences which are designed to specifically alter cognate endogenous alleles.

Methods of use for the transgenic mice of the invention are also provided herein. Knockout mice of the invention can be injected with tumor cells or treated with carcinogens to generate carcinomas. Such mice provide a biological system for assessing chemotherapy resistance as modulated by an *ABCA2* gene of the invention. Accordingly, therapeutic agents which inhibit the action of these transporters and thereby prevent efflux of beneficial chemotherapeutic agents from tumor cells may be screened in studies using *ABCA2* knock out mice.

As described above, *ABCA2*-encoding nucleic acids are also used to advantage to produce large quantities of substantially pure ABCA2 proteins, or selected portions thereof.

B. ABCA2 Proteins and Antibodies

Purified full length ABCA2 proteins, or fragments thereof, may be used to produce polyclonal or monoclonal antibodies which also may serve as sensitive detection reagents for the presence and accumulation of ABCA2 proteins (or complexes containing ABCA2 proteins) in mammalian cells. Recombinant techniques enable expression of fusion proteins containing part or all of ABCA2 proteins. The full length proteins or fragments of the proteins may be used to advantage to generate an array of monoclonal antibodies specific for various epitopes of ABCA2 proteins, thereby providing even greater sensitivity for detection of ABCA2 proteins in cells.

Polyclonal or monoclonal antibodies immunologically specific for ABCA2 proteins may be used in a variety of assays designed to detect and quantitate the proteins. Such assays include, but are not limited to: (1) flow cytometric analysis; (2) immunochemical localization of ABCA2 proteins in tumor cells; and (3) immunoblot analysis (e.g., dot blot, Western blot) of extracts from various cells. Additionally, as described above, anti-ABCA2 antibodies can be used for purification of ABCA2 proteins and any associated subunits (e.g., affinity column purification, immunoprecipitation).

From the foregoing discussion, it can be seen that ABCA2-encoding nucleic acids, *ABCA2* expressing vectors, ABCA2 proteins and anti-ABCA2 antibodies of the invention can be used to detect *ABCA2* gene expression and alter ABCA2 protein accumulation for purposes of assessing the genetic and protein interactions involved in the transport of biological and pharmacological reagents across cell membranes.

C. Methods and Kits Employing the Compositions of the Present Invention

Methods and kits encompassing the human *ABCA2*-encoding nucleic acids of the invention are also contemplated to be within the scope of the present invention.

Exemplary approaches for detecting *ABCA2* nucleic acid or polypeptides/proteins include:

a) comparing the sequence of nucleic acid in the sample with the *ABCA2* nucleic acid sequence to determine whether the sample from the patient contains mutations; or

b) determining the presence, in a sample from a patient, of the polypeptide encoded by the *ABCA2* gene and, if present, determining whether the polypeptide is full length, and/or is mutated, and/or is expressed at the normal level; or

c) using DNA restriction mapping to compare the restriction pattern produced when a restriction enzyme cuts a sample of nucleic acid from the patient with the restriction pattern obtained from normal *ABCA2* gene or from known mutations thereof; or,

d) using a specific binding member capable of binding to a *ABCA2* nucleic acid sequence (either normal sequence or known mutated sequence), the specific binding member comprising nucleic acid hybridizable with the *ABCA2* sequence, or substances comprising an antibody domain with specificity for a native or mutated *ABCA2* nucleic acid sequence or the polypeptide encoded by it, the specific binding member being labeled so that binding of the specific binding member to its binding partner is detectable; or,

e) using PCR involving one or more primers based on normal or mutated *ABCA2* gene sequence to screen for

normal or mutant *ABCA2* gene in a sample from a patient.

A "specific binding pair" comprises a specific binding member (sbm) and a binding partner (bp) which have a particular specificity for each other and which in normal conditions bind to each other in preference to other molecules. Examples of specific binding pairs are antigens and antibodies, ligands and receptors and complementary nucleotide sequences. The skilled person is aware of many other examples and they do not need to be listed here. Further, the term "specific binding pair" is also applicable where either or both of the specific binding member and the binding partner comprise a part of a large molecule. In embodiments in which the specific binding pair are nucleic acid sequences, they will be of a length to hybridize to each other under conditions of the assay, preferably greater than 10 nucleotides long, more preferably greater than 15 or 20 nucleotides long.

In most embodiments for screening for alleles giving rise to chemotherapy resistance, for example, the *ABCA2* nucleic acid in biological sample will initially be amplified, e.g. using PCR, to increase the amount of the analyte as compared to other sequences present in the sample. This allows the target sequences to be detected with a high degree of sensitivity if they are present in the sample. This initial step may be avoided by using highly sensitive array techniques that are becoming increasingly important in the art.

The identification of the full-length *ABCA2*-encoding nucleic acid, and its association with a particular chemotherapy resistance paves the way for aspects of the present invention to provide the use of materials and methods, such as are disclosed and discussed above, for establishing the presence or absence in a test sample of a variant form of the gene, in particular an allele or variant specifically associated with chemotherapy

resistance. This may be done to assess the propensity of the tumor to exhibit chemotherapy resistance.

Alternatively, *ABCA2* mutations may result in aberrant transport of endogenous biological molecules resulting in a pathological condition. For example, Rozet and colleagues have identified a spectrum of *ABCA4* gene mutations associated with autosomal recessive macular dystrophies. Rozet et al., 1998). Mutations in another ABC transporter, ABCA1, have been linked to Tangier disease (Rust et al., 1998). In a similar fashion, it is likely that mutations in the *ABCA2* gene are associated certain pathological conditions. Thus, methods to identify such mutations are contemplated to be within the scope of the present invention.

In still further embodiments, the present invention concerns immunodetection methods for binding, purifying, removing, quantifying or otherwise generally detecting biological components. The encoded proteins or peptides of the present invention may be employed to detect antibodies having reactivity therewith, or, alternatively, antibodies prepared in accordance with the present invention, may be employed to detect the encoded proteins or peptides. The steps of various useful immunodetection methods have been described in the scientific literature, such as, e.g., Nakamura et al. (1987).

In general, the immunobinding methods include obtaining a sample suspected of containing a protein, peptide or antibody, and contacting the sample with an antibody or protein or peptide in accordance with the present invention, as the case may be, under conditions effective to allow the formation of immunocomplexes.

The immunobinding methods include methods for detecting or quantifying the amount of a reactive component in a sample, which methods require the

detection or quantitation of any immune complexes formed during the binding process. Here, one would obtain a sample suspected of containing an *ABCA2* gene encoded protein, peptide or a corresponding antibody, and contact the sample with an antibody or encoded protein or peptide, as the case may be, and then detect or quantify the amount of immune complexes formed under the specific conditions.

In terms of antigen detection, the biological sample analyzed may be any sample that is suspected of containing the ABCA2 antigen, such as a tumor tissue section or specimen, a homogenized tissue extract, an isolated cell, a cell membrane preparation, separated or purified forms of any of the above protein-containing compositions.

Contacting the chosen biological sample with the protein, peptide or antibody under conditions effective and for a period of time sufficient to allow the formation of immune complexes (primary immune complexes) is generally a matter of simply adding the composition to the sample and incubating the mixture for a period of time long enough for the antibodies to form immune complexes with, i.e., to bind to, any antigens present. After this time, the sample-antibody composition, such as a tissue section, ELISA plate, dot blot or Western blot, will generally be washed to remove any non-specifically bound antibody species, allowing only those antibodies specifically bound within the primary immune complexes to be detected.

In general, the detection of immunocomplex formation is well known in the art and may be achieved through the application of numerous approaches. These methods are generally based upon the detection of a label or marker, such as any radioactive, fluorescent, biological or enzymatic tags or labels of standard use in the art. U.S.

Patents concerning the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241, each incorporated herein by reference. Of course, one may find additional advantages through the use of a secondary binding ligand such as a second antibody or a biotin/avidin ligand binding arrangement, as is known in the art.

In one broad aspect, the present invention encompasses kits for use in detecting expression of *ABCA2*-encoding nucleic acids in biological samples, including biopsy samples. Such a kit may comprise one or more pairs of primers for amplifying nucleic acids corresponding to the *ABCA2* gene. The kit may further comprise samples of total mRNA derived from tissues expressing the *ABCA2*-encoding nucleic acid of the invention, to be used as controls. The kit may also comprise buffers, nucleotide bases, and other compositions to be used in hybridization and/or amplification reactions. Each solution or composition may be contained in a vial or bottle and all vials held in close confinement in a box for commercial sale. In a further embodiment, the invention encompasses a kit for use in detecting ABCA2 proteins in chemotherapy resistant cancer cells comprising antibodies specific for ABCA2 proteins encoded by the *ABCA2* nucleic acids of the present invention.

Another aspect of the present invention comprises screening methods employing host cells expressing an *ABCA2*-encoding nucleic acid of the invention. An advantage of having discovered the complete coding sequence of *ABCA2* is that cell lines that overexpress ABCA2 can be generated using standard transfection protocols. Cells that overexpress the complete cDNA will also harbor the complete proteins a feature that is essential for

assessing biological activity of the protein. The overexpressing cell lines will be useful in several ways: 1)The drug sensitivity of overexpressing cell lines can be tested with a variety of known anticancer agents in order to determine the spectrum of anticancer agents for which the transporter confers resistance; 2)The drug sensitivity of overexpressing cell lines can be used to determine whether newly discovered anticancer agents are transported out of the cell by one of the discovered transporters; 3)Overexpressing cell lines can be used to identify potential inhibitors that reduce the activity of the transporters. Such inhibitors are of great clinical interest in that they may enhance the activity of known anticancer agents, thereby increasing their effectiveness. Reduced activity will be detected by restoration of anticancer drug sensitivity, or by reduction of transporter mediated cellular efflux of anticancer agents. In vitro biochemical studies designed to identify reduced transporter activity in the presence of potential inhibitors can also be performed using membranes prepared from overexpessing cell lines; and 4)Overexpressing cell lines can also be used to determine whether pharmaceutical agents that are not anticancer agents are transported out of the cell by the transporters.

The following protocols are provided to facilitate the practice of the present invention.

Isolation of ABCA2 cDNA

We cloned the *ABCA2* cDNA using a variety of library screening and PCR-based approaches. As previously reported (Laing et al., 1998), we first isolated 1.75 kb of *ABCA2* cDNA by PCR using primers designed against the expressed sequence tag representing human *ABCA2* (EST0600) and mouse *abca2* cDNA (GenBank Accession number X75927).

Our initial library screening experiments were performed using a ready-made random and oligo(dT)-primed human fetal brain cDNA library generated in lambda ZAP II (Stratagene, La Jolla, CA). This library was chosen because *ABCA2* expression in mouse is most pronounced in brain (Luciani et al., 1994). The library was probed with a ^{32}P-labeled (Prime-it II Random Primer Labeling Kit, Stratagene, La Jolla, CA) 490 bp PCR fragment that we amplified from the 5' end of the 1.75 kb ABCA2 fragment. Screening was performed according to the manufacturer's instructions. In brief, XL1-Blue bacteria were incubated with different dilutions of library and plated onto LB plates to titer the library. After plating 50,000 pfu /150 mm plate and subsequent incubations, colonies were transferred to nitrocellulose filters, which were then denatured, neutralized and washed. DNA was cross-linked to the filters and they were then prehybridized for at least 2 h at 65°C and hybridized overnight. After post-hybridization washing, filters were autoradiographed to identify positives. The four longest *ABCA2* clones were designated as λ15B, λ17B, λ5A, λ9A. See Figure 1.

5' RACE using nested-PCR was performed using human brain Marathon-Ready adapter-ligated cDNA as template (Clontech, Palo Alto, CA). The first round of PCR used adapter primer 1 (AP1) 5'-CCATCCTAATACGACTCACTATAGGGC-3' (forward; Sequence I.D. No. 3) and *ABCA2* specific 5'-TGAGTTTGTCCACGCAGACAACCAGAG-3' (reverse; Sequence I.D. No. 4); adapter primer 2 (AP2) 5'-ACTCACTATAGGGCTCGAGCGGC-3' (forward; Sequence I.D. No. 5) with *ABCA2* specific 5'-CCAGCTCCACTCCCAGGCTTCTG-3' (reverse; Sequence I.D. No. 6). The products were ligated into the pT-Adv plasmid (Clontech, Palo Alto, CA) and ligation extraction products used to transform TOPO10' cells. Based on additional 5' sequence obtained

from this approach, (clone 35, Fig. 1), the following primers were designed for subsequent 5'RACE: AP1 (forward) and 5'-TGAGTTTGTCCACGCAGACAACCAGAG-3' (reverse; Sequence I.D. No. 7); second round PCR used AP2 (forward) and 5'-CCAGCTCCACTCCCAGGCTTCTG-3' (reverse; Sequence I.D. No. 8). The longest clone isolated, designated 208 was ~900 bp (Fig. 1).

To obtain the entire 5' end sequence, we constructed a 5' *ABCA2* cDNA library from high quality human brain poly (A)+ RNA (Clontech, Palo Alto, CA) using Marathon cDNA Amplification Kit (Clontech, Palo Alto, CA). Gene specific primer was used for first strand synthesis. Second strand synthesis and creation of blunt ends was followed by DNA ligation with adapters provided with the kit, according to the manufacturer's protocol. The library was then PCR amplified using AP1 and 5'-CCACTGGG CAGCGAGAAGTTGTC-3' (Sequence I.D. No. 9) followed by amplification with nested *ABCA2* primer 5'-GAAGCTGGAGTTCTGGCGGATCT-3' (Sequence I.D. No. 10) and adapter primer, AP2. Reaction products were cloned into pCR-XL-TOPO plasmid (Invitrogen, Carlsbad, CA) and transformed into DH5 α cells. Figure 1 indicates the location of the two longest clones, designated 65 and 119.

For 3'RACE, we used: 5'CAGACCACACTGGACAATGTGTTCGTG3' (forward; Sequence I.D. No. 11) and AP1 (reverse); 5' TCATCAGCTTCGAGGAGGAGCGG3' (forward; Sequence I.D. No. 12) and AP2 (reverse). Only short, 300 bp fragments were isolated (data not shown).

Full length *ABCA2* cDNA was assembled from 4 fragments (A-D). Fragments A and B were obtained by PCR from human brain cDNA library using the following primers: 5'ATAAGCTTGCTGAGGCGGCGGAGCGTGGC3' (Sequence I.D. No. 13) and 5'CCACTGGGCGAGAAGTTGTC3' (Sequence I.D. No. 14) for fragment A, and 5'CCTCATTTTCCCCTACAACC3'

(Sequence I.D. No. 15) and 5'ACCTGCTCCATCTTGCTGCTGAACAC, (Sequence I.D. No. 16) for fragment B. Fragment C was directly obtained by restriction digest of KIA1065 clone (kindly provided by Dr Takahiro Nagase from Kazusa DNA Research Institute). Fragment D was obtained by PCR from KIA1065 clone using 5'CAGCGGCGGCAACAAGCGGAA3' (Sequence I.D. No. 17) and 5'GGTGAATTCGGCAGGCACTGGGGGACTTGT3' (Sequence I.D. No. 18) primers. PCR products were initially cloned into pCR-XL-TOPO cloning vector. Fragment A was excised by Hind III and Sal I digest and subcloned into pCR-XL-TOPO clone containing fragment B. Fragment D was excised by Kpn I and EcoR I digestion and subcloned into corresponding sites of (A+B)pCR-XL-TOPO construct. Finally fragment C was cloned into Kpn I site of (A+B+D) pCR-XL-TOPO clone. The full length *ABCA2* cDNA was excised from pCR-XL-TOPO-*ABCA2* by Hind III and EcoR I digestion and subloned into corresponding sites of pcDNA (3.1+) vector (Invitrogen, Carlsbad, CA). That clone has been designated as pcDNA(3.1+)-*ABCA2*.

pEGFP-*ABCA2* clone was constructed in the following way: start codon of *ABCA2* was modified using PCR (primers used were 5'TAGTACTCCTTGGGCTTCCTGCACCAGC3'(Sequence I.D. No. 19) and 5'CCAGGGCAGATGAGGGACCAAAGA3' (Sequence I.D. No. 20)), and resulting clone inserted into ScaI and EcoRI sites of pEGFP-C3 vector (Clontech, Palo Alto, CA). All PCR products were verified by double-stranded DNA sequencing.

Mapping of transcription start site

5' RACE was used to map the start site of the *ABCA2* transcript. Reverse transcription of total brain RNA (Clontech, Palo Alto) was performed using antisense gene specific primer, 5'CATCCAGCAGGTCCCCCAGAAGC 3' (Sequence I.D. No. 21) and was followed by RNase H treatment. The

first strand synthesis product was subjected to dC tailing reactions with terminal deoxynucleotidyl transferase. The first round of PCR amplification was then performed using 5' RACE anchor primer 5'GGCCACGCGTCGACTAGTACGGGIIGGGIIGGGIIG3' (Sequence I.D. No. 22) and gene-specific antisense primer 5'AAACAGGTTGCCCTTCCTCCACCAC3' (Sequence I.D. No. 23). A second round of PCR amplification was performed with universal amplification primer 5'GGCCACGCGTCGACTAGTAC3' (Sequence I.D. No. 24) and gene specific antisense primer 5'ACAGCGATTGCATGACAGGCAG3' (Sequence I.D. No. 25). A single ~ 300 bp product was obtained, and after purification it was cloned and sequenced.

Northern blot analysis

Samples of the total RNA from a selection of NCI panel human tumor cell lines were provided by Dr. Anne Monks (Monks et al., 1991). 10 µg samples were fractionated on formaldehyde agarose gels, transferred to nylon membranes (GeneScreen, NEN, Boston, MA) and hybridized by standard protocols. ^{32}P radioactive labeling of a gel-purified 1.75 kb *ABCA2* probe to high specific activity was performed by random priming kit (Prime-It 11 Random Primer Labeling Kit, Stratagene, La Jolla, CA).

A multiple human tissue poly(A)+RNA dot blot (MTE Array, Clontech, Palo Alto, CA), and Multiple Tissue Northern Blot (MTN, Clontech, Palo, Alto, CA) were hybridized with a 870 bp probe that was PCR amplified using the following primers: 5' AGGGAGCTGGCTACACCGACG 3' (forward; Sequence I.D. No. 26) and 5'CGCCTGTGACCACCCGCATCT 3' (reverse; Sequence I.D. No. 27). Portions were biotin-labeled (for MTE Array screen) or ^{32}P radioactive labeled (for MTN screen) according to the random primer labeling method. Non-radioactive

detection was performed with North 2 South Chemiluminescent Detection system (Pierce, Rockford, IL). The signal intensities were quantified with NIH Image software.

ABCA2 sequence analysis

Nucleotide sequencing was performed with an ABI 377 DNA sequencer. The sequences were assembled in the Sequencher program (Gene Codes Corporation, Ann Arbor, MI). Protein computer analyses were performed with the Genetics Computer Group Package version 9.1 (Madison, WI), and McVector (Oxford Molecular).

The following example is provided to illustrate an embodiment of the invention. It is not intended to limit the invention in any way.

EXAMPLE I

Cloning of human *ABCA2*

The *ABCA2* transporter was cloned by initially screening an oligo(dT) and random primed human fetal brain cDNA library probed with a PCR-amplified 1.75 kb fragment of *ABCA2* (Laing et al., 1998). Out of four isolated clones, the longest, lambda λ9A, was 4.8 kb in length (Fig. 1). This fragment showed 86% nucleotide identity to the 4.7 kb of available mouse *ABCA2* with the least homology in the last 300 bp, and no predicted in-frame stop codon. Initial 5' RACE yielded another ~1.4 kb of sequence at the 5 'end of *ABCA2* cDNA from the longest overlapping clones, 35 and 208 (Fig. 1). We created our own human brain Marathon cDNA using gene specific primers near the 5' end in order to obtain an ATG start codon with apparent Kozak sequence. The two longest amplified products, 65 and 119, both contained the same 5' UTR and the ATG start in standard Kozak

context (Kozak, 1991). 5' RACE was performed to map the transcriptional start site and yielded a single band. Thus, the major transcriptional start site is at a cytosine, 50bp upstream of the translational start site.

For the 3' RACE experiment, we used gene specific primers upstream from the predicted stop codon, (based on mouse ORF) (Luciani et al., 1994) and generated a number of 300-400 bp fragments containing putative stop codons and an additional ~300 bp of 3'UTR (data not shown). In addition, an interim BLAST search of the sequence of the λ9A clone (Fig. 1), returned a brain clone (GenBank number AB028985), with high identity to the last ~4.7 kb of this clone and provided in addition to 300 bp, another ~400 bp of 3'UTR (Fig. 1). The total sequence assembled resulted in 8056 bp *ABCA2* cDNA, with 7308 bp corresponding to ORF, 50 bp 5'UTR and 698 bp 3'UTR (Fig. 1). Comparison of the nucleotide sequences of ABCA1-related proteins to *ABCA2* showed that these transporters share 51-52% identity.

Primary Structure of ABCA2

Nucleotide analysis revealed that the ABCA2 open reading frame was comprised of 7308 bp and encoded a 2436 amino acid protein with a predicted molecular weight of 270 kDa and a pI of 6.4. See Figure 2. Comparison of the ABCA2 primary structure with several other human ABC-transporters, showed that ABCA2 shares 45.4% amino acid identity with ABCA1 and slightly less with ABCA4 (38.5%) and hABCA3 (38.2%) (Table 1). The protein is less similar to other well characterized ABC transporters, such as MDR1 (33% identical) and MRP (25% identical). Homology comparison with the partial mouse ABCA2 sequence (Luciani et al., 1994) revealed 94.4% amino acid identity within the published region. The amino terminal of ABCA2 shares high identity with the

amino terminal of ABCA4 and ABCA3 (65-70% in the first 20 amino acids), although homology with these proteins drops significantly in adjacent domains of ABCA2.

The ATP-binding cassettes of ABCA2 contain conserved Walker A and B ATP-binding motifs together with the signature sequence of ABC transporters (Fig. 3). The amino terminal ABC of ABCA2 is most identical to ABCA1 (70.6%) and slightly less to that of ABCA4 (65.2%) and ABCA3 (58.2%). The carboxy-terminal has highest identity with ABCA4 (67.8%) and ABCA1 (66.1%) and somewhat less with ABCA3 (60.7%).

The ABCA2 protein is a full-size transporter that contains a tandem repeat of the recognizable hydrophobic domain with six transmembrane helices followed by highly conserved ATP-binding cassettes. Despite the apparent structural symmetry, there is very little sequence homology between the two halves of the predicted ABCA2 protein, as seen by dot matrix analysis (Fig. 2D). Structurally, the hydrophobic domain spacing is reminiscent of that found in ABCA4 (Figs. 2 B, 2C). The cytosolic N-terminus is immediately followed by the first transmembrane segment and a long extra-cytosolic loop. The long hydrophilic linker portion of the protein (~700 amino acids) is separated into two halves by a highly hydrophobic domain. This region of the protein, as suggested by Luciani and colleagues, may correspond to a putative regulatory domain similar to the one found in CFTR. The potential sites of N-glycosylation are concentrated in two regions, with the first fifteen on the large extracellular loop and the second six between the HHD and the 7th transmembrane segment. While these post-translation modifications are highly probable in the first region, glycosylation at the second region may be more hypothetical, based on the present understanding of ABC1-like proteins (Azarian and Travis, 1997). While

some groups (Azarian and Travis, 1997; Rozet et al., 1998) favor a structure in which a highly hydrophobic domain (HHD) is followed by a cytoplasmic segment, thereby negating glycosylation within the second region, Illing and colleagues (Illing et al., 1997) based on peptide analysis, predicted a model in which a glycosylated extracellular loop is present between HHD and the seventh transmembrane segment. Thus, it is possible that the HHD may represent an actual full-span transmembrane segment.

A number of potential phosphorylation sites were also apparent, including, protein kinase C, casein kinase, tyrosine kinase and cAMP dependent-protein kinase. Of particular interest to our earlier observation of the potential role of ABCA2 in the transport of steroids (Laing et al., 1998), a lipocalin signature (GQSRKLDGGWLKV) was identified at position 1424 within the putative regulatory domain and close to the HHD. This motif is characteristic of small lipocalin proteins that transport lipids, steroids, bilins and retinoids (Flower, 1996).

Table 1.

Comparison of amino acid sequences (% identity) between members of ABC1 subfamily[a]

	ABCA2	ABCA1	ABCA4	ABCA3	MusABCA2
ABCA2	-	45.4 (70.6/66/1)	38.5 (65.2/67.8)	38.2 (58.2/60.7)	94.4 (98.9/95.1)
ABCA1	45.4 (70.6/66.1)	-	51.8 (69.0/73.2)	40.4 (62.0/55.2)	51.7 (70.1/66.1)
ABCA4	38.5 (65.2/67.8)	51.8 (69.0/73.2)	-	36.8 (60.4/59.0)	43.5 (64.7/67.2)
ABCA3	38.2 (58.2/60.7)	40.4 (62.0/55.2)	36.8 (60.4/59.0)	-	42.0 (58.3/60.7)
MusABCA2	94.4 (98.9/95.1)	51.7 (70.1/66.1)	43.5 (64.7/67.2)	42.0 (58.3/60.7)	-

[a]GAP function in GCG software was used to obtain data. Numbers in parentheses represent percent identity of ATP-binding cassette 1 and 2 (ABCA1/ABCA2).

Human ABCA2 expression pattern

Northern analysis of *ABCA2* message shows that the *ABCA2* transcript is ~8 kb in length (Fig. 4). Of the 12 tissues analyzed, *ABCA2* expression is highest in the brain. The dot blot analysis confirmed that *ABCA2* was most prevalent in the central nervous system (Fig. 5). The highest expressing regions of CNS are spinal cord, corpus callosum, medulla oblongata and thalamus. This expression was higher in adult brain than fetal and there were also significant levels in thyroid gland and lymphoid tissue, such as appendix and spleen. The expression levels in other tissues were rather low. In the NCI cell line panel of tumors, there was a more ubiquitous pattern (Table 2). The SNB-75 cell line was the highest among the CNS; however, the presence of measurable *ABCA2* transcript in most of these cell lines may be a characteristic of the transformed phenotype.

Table 2

ABCA2 mRNA in Partial NCI Cell Line Panel

Cell Line	*ABCA2*	Cell Line	*ABCA2*
Melanoma		**Leukemic**	
SK-MEL-28	+	SR	++
SK-MEL-5	++	RPMI-8226	+
UACC-257	++	HL60-TB	+
UACC-62	++	CCRF-CEM	+
M14	+++		
MALME-3M	+		

		Breast	
	LOXIMVI	++	
		MCF7	++++
	CNS	NCI/ADR	++
	SF-295	++	
	SF-539	+	
	SNB-19	++	**Colon**
	SNB-75	++++	
	U251	++	HCC-2998 +
		HCT-116	+
	LUNG	HCT-15	+
		SW	++
	A549	++	
	NCI-H23	++++	
	NCI-H460	++	**Renal**
	NCI-H522	+++	
	EKVX	++++	786-0 +++
	HOP-62	+	A498 +++
	HOP-92	++	CAKI-1 +
		JK-10	++
	Prostate	UO-31	+
	PC-3	++	**Ovarian**
		OV-CAR-3	++
		OV-CAR-5	++
		OV-CAR-8	++
		IGR-OV1	++

In summary, we have isolated the full length human *ABCA2* cDNA and performed molecular and primary structure analysis on the encoded ABCA2 protein transporter. Based on the degree of amino acid identity, ABCA2 can be regarded as an orthologue of mouse ABCA2 (Luciani et al.,

1994). This protein is closely related to members of the ABCA1-subfamily of transporters (ABCA1, ABCA4 and ABCA3) and more distantly related to MDR1, MRP1 and CFTR.

The availability of the *ABCA2* cDNA and its encoded protein facilitate the development of novel therapeutic agents which may be efficacious in the treatment of cancer and other disorders which result from the aberrant transport of molecules across cell membranes.

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While certain of the preferred embodiments of the present invention have been described and specifically exemplified above, it is not intended that the invention be limited to such embodiments. Various modifications may be made thereto without departing from the scope and spirit of the present invention, as set forth in the following claims.

What is claimed is:

1. An isolated nucleic acid molecule having the sequence of SEQ ID NO:1, said nucleic acid molecule comprising a nucleotide sequence encoding a human ABCA2 transporter protein about 2436 amino acids in length, said encoded transporter protein comprising a multi-domain structure including a multiplicity of glycosylation and phosphorylation sites, a lipocalin signature motif, nucleotide binding folds having Walker A and B ATP binding sites, and a plurality of membrane spanning helices.

2. The nucleic acid molecule of claim 1, which is DNA.

3. The DNA molecule of claim 2, which is a cDNA comprising a sequence approximately 7.3 kilobase pairs in length that encodes said human ABCA2 transporter protein.

4. The DNA molecule of claim 2, which is a gene comprising introns and exons, the exons of said gene specifically hybridizing with the nucleic acid of Sequence I.D. No. 1, and said exons encoding said *ABCA2* transporter protein.

5. An isolated RNA molecule transcribed from the nucleic acid of claim 1.

6. The nucleic acid molecule of claim 1, wherein said sequence encodes a human ABC2 transporter protein having an amino acid sequence selected from the group consisting of Sequence I.D. No. 2 and amino acid sequences encoded by natural allelic variants of said sequence.

7. The nucleic acid molecule of claim 6, which comprises Sequence I.D. No. 1.

8. An antibody immunologically specific for the protein encoded by the nucleic acid of claim 1.

9. An antibody as claimed in claim 8, said antibody being monoclonal.

10. An antibody as claimed in claim 8, said antibody being polyclonal.

11. A plasmid comprising a nucleotide sequence having the sequence of Sequence I.D. No. 1.

12. A vector comprising a nucleotide sequence having the sequence of Sequence I.D. No. 1.

13. A retroviral vector comprising a nucleotide sequence having the sequence of Sequence I.D. No. 1.

14. A host cell comprising a nucleic acid molecule having a sequence of Sequence I.D. No. 1.

15. A host cell as claimed in claim 14, wherein said host cell is selected from the group consisting of bacterial, fungal, mammalian, insect and plant cells.

16. A host cell as claimed in claim 14, wherein said nucleic acid is provided in a plasmid and is operably linked to mammalian regulatory elements which confer high expression and stability of mRNA transcribed from said nucleic acid.

17. A host cell as claimed in claim 14, wherein said nucleic acid is provided in a plasmid and is operably linked to mammalian regulatory control elements in reverse anti-sense orientation.

18. A host animal comprising a nucleic acid molecule having the sequence of Sequence I.D. No. 1.

19. A host animal as claimed in claim 18, wherein said animal harbors a homozygous null mutation in its endogenous *ABCA2* gene wherein said mutation has been introduced into said mouse or an ancestor of said mouse via homologous recombination in embryonic stem cells, and further wherein said mouse does not express a functional mouse ABC2 protein.

20. The transgenic mouse of claim 18, wherein said mouse is fertile and transmits said null mutation to its offspring.

21. The transgenic mouse of claim 18, wherein said null mutation has been introduced into an ancestor of said mouse at an embryonic stage following microinjection of embryonic stem cells into a mouse blastocyt.

22. A method for screening a test compound for inhibition of human ABCA2 mediated transport, comprising:

a) providing a host cell expressing a human *ABCA2*-encoding nucleic acid having a sequence of Sequence I.D. No. 1;

b) contacting said host cell with a compound suspected of inhibiting human ABC2-mediated transporter activity; and

c) assessing inhibition of transport mediated by said compound.

23. A method as claimed in claim 22, wherein inhibition of human ABCA2 mediated transport is indicated by restoration of anticancer drug sensitivity.

24. A method as claimed in claim 22, wherein said inhibition of human ABCA2 mediated transport is indicated by a reduction of transporter mediated cellular efflux of anticancer agents.

25. A kit for detecting the presence of human *ABCA2*-encoding nucleic acids in a sample, comprising:

a) oligonucleotide primers specific for amplification of human *ABCA2*-encoding nucleic acids;

b) polymerase enzyme;

c) amplification buffer; and

d) human *ABCA2* specific DNA for use as a positive control.

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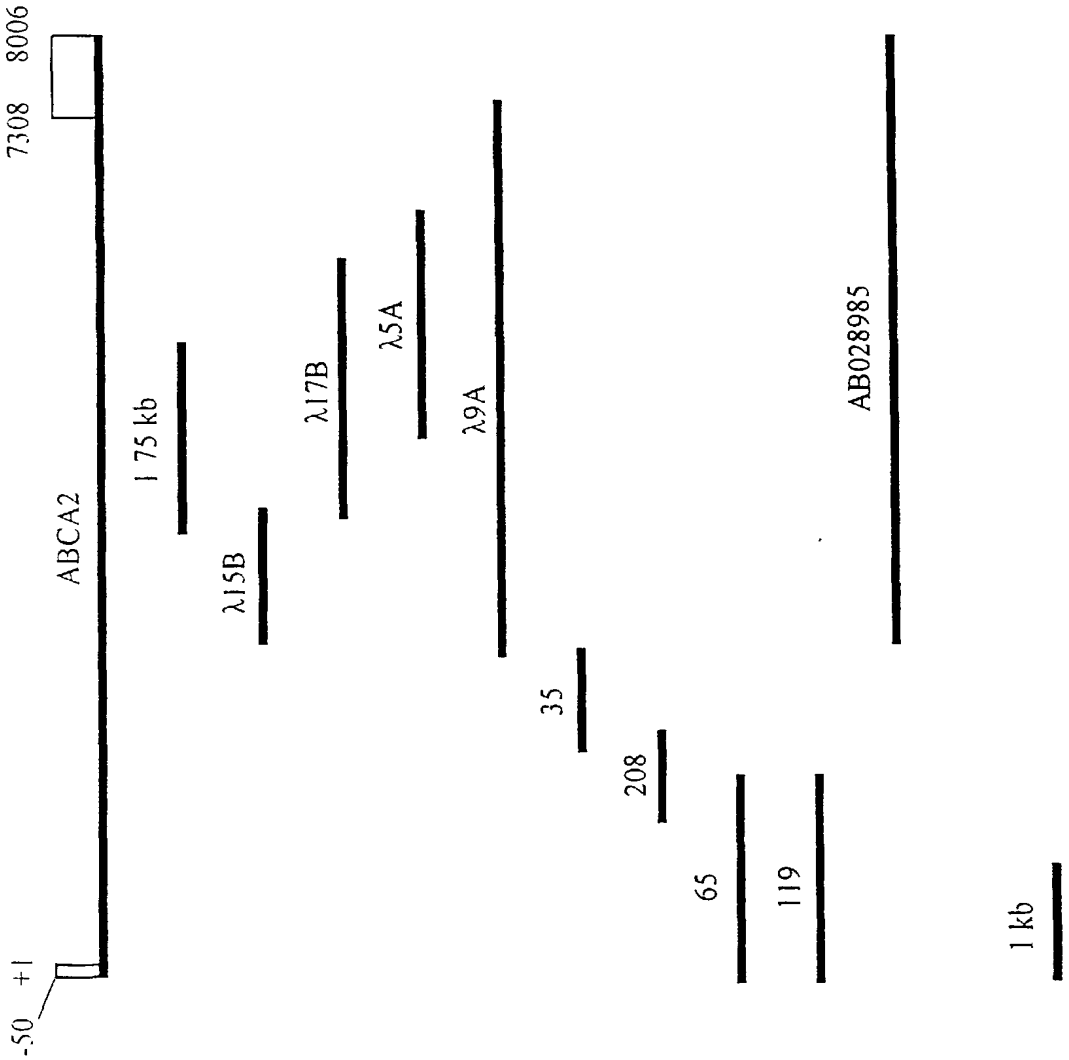
(54) Title: NUCLEIC ACID ENCODING HUMAN ABCA TRANSPORTER 2 (ABCA2) AND METHODS OF USE THEREOF

(57) Abstract:

Figure 1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ABCA2
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+1
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λ15B
λ17B
λ5A
λ9A
35
208
65
119
AB028985
1 kb

Figure 2A

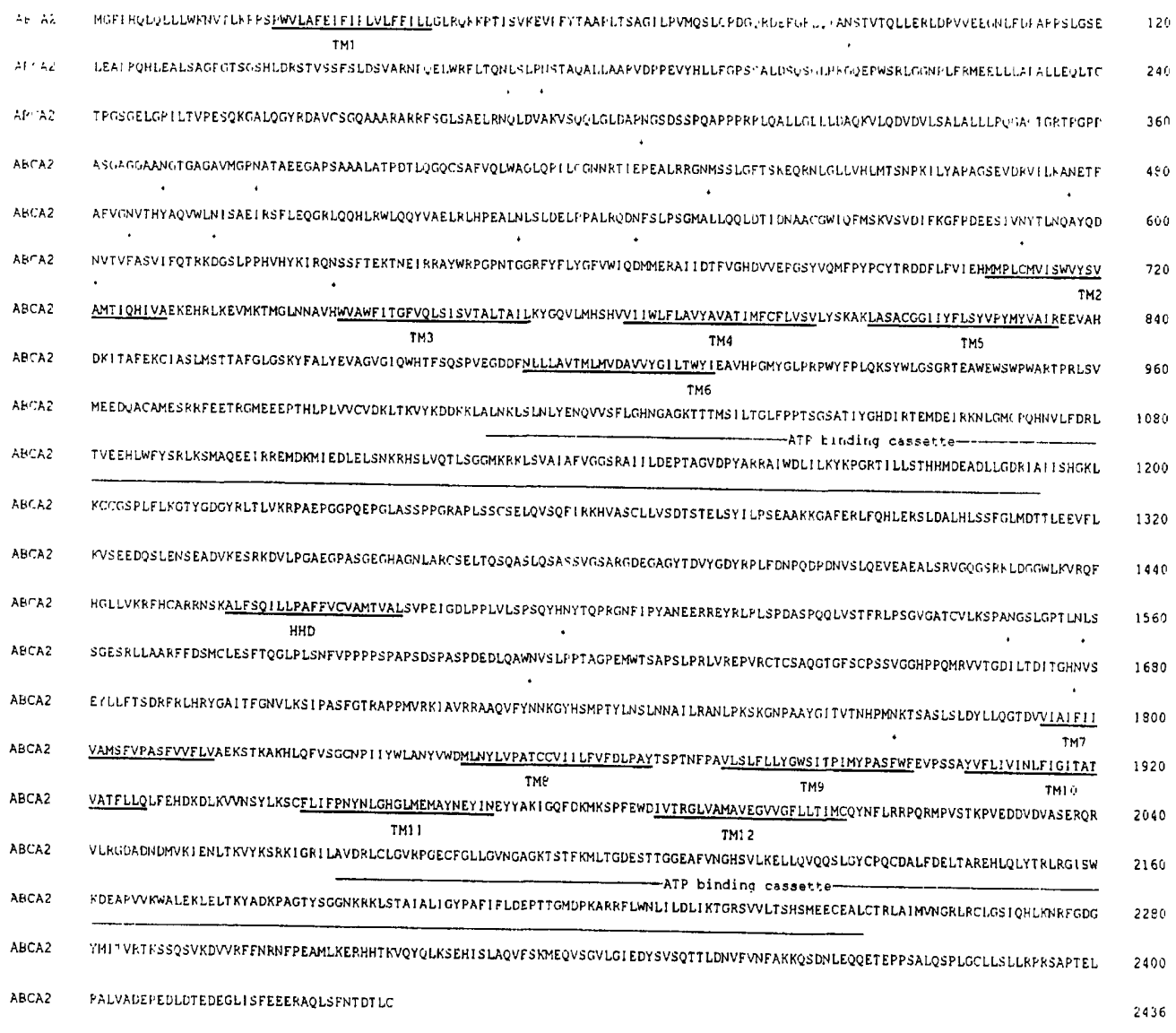

TM1
TM2
TM3
TM4
TM5
TM6
ATP binding cassette
HHD
TM7
TM8
TM9
TM10
TM11
TM12
ATP binding cassette

Figure 2B

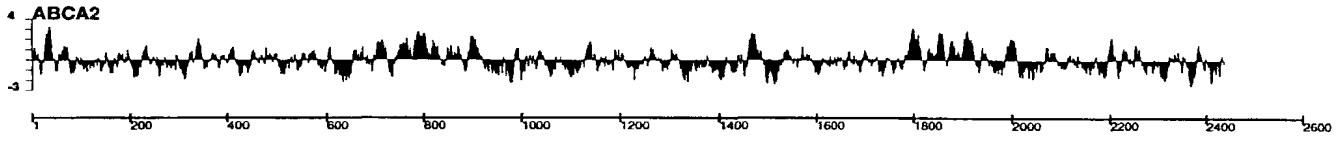
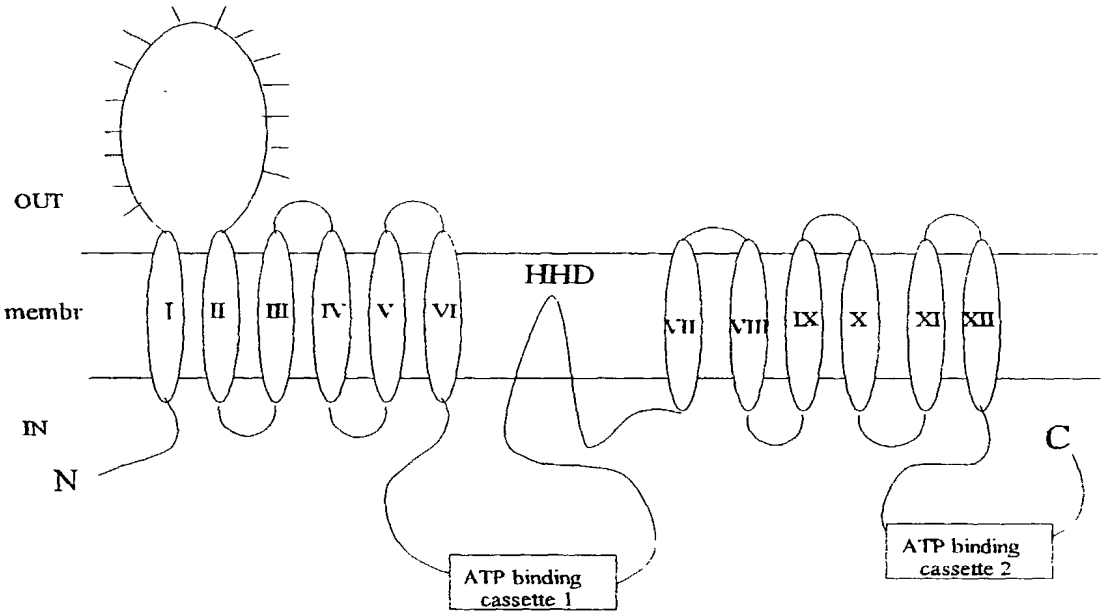

4
ABCA2
-3
1
200
400
600
800
1000
1200
1400
1600
1800
2000
2200
2400
2600

Figure 2C


OUT
membr
IN
N
C
I
II
III
IV
V
VI
HHD
VII
VIII
IX
X
XI
XII
ATP binding cassette 1
ATP binding cassette 2

200
600
1000
1400
1800
2200
ABCA2
2200
1800
1400
1000
600
200
ABCA2

Figure 2D

Figure 3A

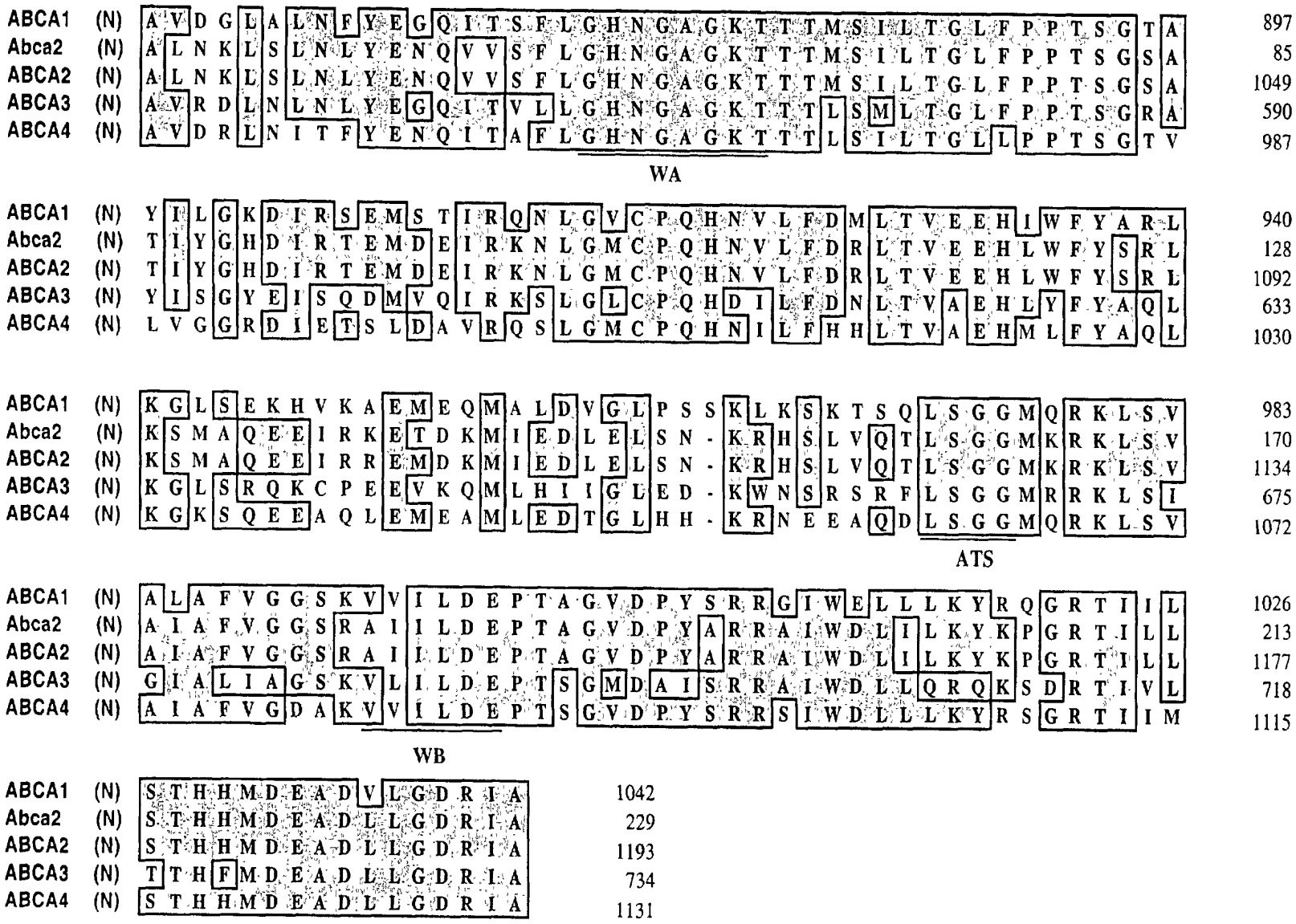
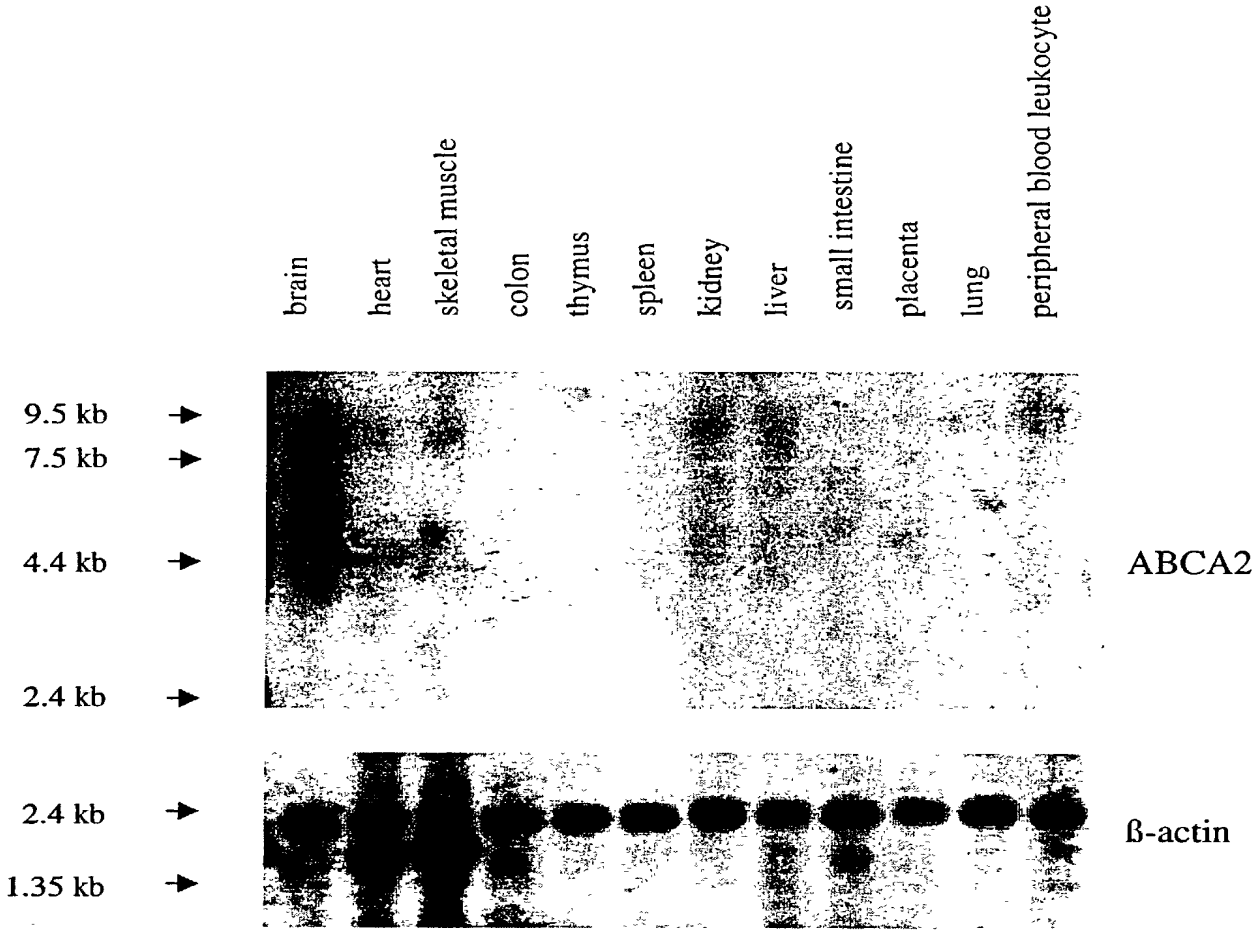

ABCA1 (N) AVDGLALNFYEGQITSFLGHNGAGKTTTMSILTGLFPPTSGTA 897
Abca2 (N) ALNKLSLNLYENQVVSFLGHNGAGKTTTMSILTGLFPPTSGSA 85
ABCA2 (N) ALNKLSLNLYENQVVSFLGHNGAGKTTTMSILTGLFPPTSGSA 1049
ABCA3 (N) AVRDLNLNLYEGQITVLLGHNGAGKTTTLSMLTGLFPPTSGRA 590
ABCA4 (N) AVDRLNITFYENQITAFLGHNGAGKTTTLSILTGLLPPTSGTV 987
WA
ABCA1 (N) YILGKDIRSEMSTIRQNLGVCPQHNVLFDMLTVEEHIWFYARL 940
Abca2 (N) TIYGHDIRTEMDEIRKNLGMCPQHNVLFDRLTVEEHLWFYSRL 128
ABCA2 (N) TIYGHDIRTEMDEIRKNLGMCPQHNVLFDRLTVEEHLWFYSRL 1092
ABCA3 (N) YISGYEISQDMVQIRKSLGLCPQHDILFDNLTVAEHLYFYAQL 633
ABCA4 (N) LVGGRDIETSLDAVRQSLGMCPQHNILFHHLTVAEHMLFYAQL 1030
ABCA1 (N) KGLSEKHVKAEMEQMALDVGLPSSKLKSKTSQLSGGMQRKLSV 983
Abca2 (N) KSMAQEEIRKETDKMIEDLELSN-KRHSLVQTLSGGMKRKLSV 170
ABCA2 (N) KSMAQEEIRREMDKMIEDLELSN-KRHSLVQTLSGGMKRKLSV 1134
ABCA3 (N) KGLSRQKCPEEVKQMLHIIGLED-KWNSRSRFLSGGMRRKLSI 675
ABCA4 (N) KGKSQEEAQLEMEAMLEDTGLHH-KRNEEAQDLSGGMQRKLSV 1072
ATS
ABCA1 (N) ALAFVGGSKVVILDEPTAGVDPYSRRGIWELLLKYRQGRTIIL 1026
Abca2 (N) AIAFVGGSRAIILDEPTAGVDPYARRAIWDLILKYKPGRTILL 213
ABCA2 (N) AIAFVGGSRAIILDEPTAGVDPYARRAIWDLILKYKPGRTILL 1177
ABCA3 (N) GIALIAGSKVLILDEPTSGMDAISRRAIWDLLQRQKSDRTIVL 718
ABCA4 (N) AIAFVGDAKVVILDEPTSGVDPYSRRSIWDLLLKYRSGRTIIM 1115
WB
ABCA1 (N) STHHMDEADVLGDRIA 1042
Abca2 (N) STHHMDEADLLGDRIA 229
ABCA2 (N) STHHMDEADLLGDRIA 1193
ABCA3 (N) TTHFMDEADLLGDRIA 734
ABCA4 (N) STHHMDEADLLGDRIA 1131

Figure 3B

ABCA1 (C) 1909
Abca2 (C) 1149
ABCA2 (C) 2111
ABCA3 (C) 1439
ABCA4 (C) 1995
WA
ABCA1 (C) 1952
Abca2 (C) 1192
ABCA2 (C) 2154
ABCA3 (C) 1482
ABCA4 (C) 2038
ABCA1 (C) 1995
Abca2 (C) 1235
ABCA2 (C) 2197
ABCA3 (C) 1525
ABCA4 (C) 2081
ATS
ABCA1 (C) 2038
Abca2 (C) 1278
ABCA2 (C) 2240
ABCA3 (C) 1568
ABCA4 (C) 2124
WB
ABCA1 (C) 2050
Abca2 (C) 1290
ABCA2 (C) 2252
ABCA3 (C) 1580
ABCA4 (C) 2136

Figure 4


brain
heart
skeletal muscle
colon
thymus
spleen
kidney
liver
small intestine
placenta
lung
peripheral blood leukocyte
9.5 kb
7.5 kb
4.4 kb
2.4 kb
ABCA2
2.4 kb
1.35 kb
ß-actin

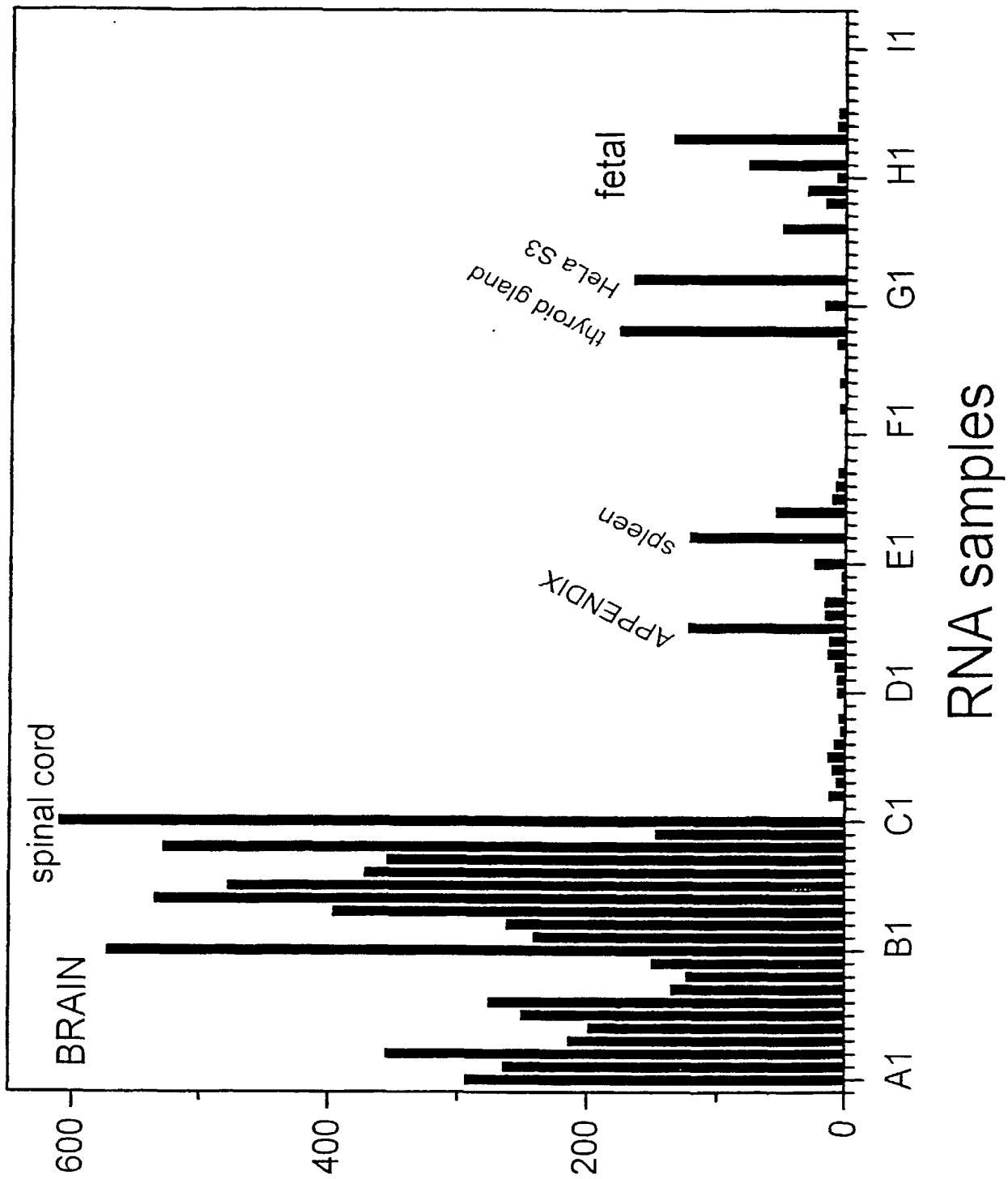

Densitometry units/ng of RNA
600
400
200
0
A1
B1
C1
D1
E1
F1
G1
H1
I1
RNA samples
BRAIN
spinal cord
APPENDIX
spleen
thyroid gland
HeLa S3
fetal

Figure 5

Figure 6A

hABC2 nucleotide sequence (SEQ ID NO: 1)

CCGCGGCGCTGAGGCGGCGGAGCGTGGCCCCGCCATGGGCTTCCTGCACCAGCTGCAGCTGCTGCTCTGGAAGAACGTGA
CGCTCAAACGCCGGAGCCCGTGGGTCCTGGCCTTCGAGATCTTCATCCCCCTGGTGCTGTTCTTTATCCTGCTGGGGCTG
CGACAGAAGAAGCCCACCATCTCCGTGAAGGAAGTCCCCTTCTACACAGCGGCGCCCCTGACGTCTGCCGGCATCCTGCC
TGTCATGCAATCGCTGTGCCCGGACGGCCAGCGAGACGAGTTCGGCTTCCTGCAGTACGCCAACTCCACGGTCACGCAGC
TGCTTGAGCGCCTGGACCGCGTGGTGGAGGAAGGCAACCTGTTTGACCCAGCGCGGCCCAGCCTGGGCTCAGAGCTCGAG
GCCCTACGCCAGCATCTGGAGGCCCTCAGTGCGGGCCCGGGCACCTCGGGGAGCCACCTGGACAGATCCACAGTGTCTTC
CTTCTCTCTGGACTCGGTGGCCAGAAACCCGCAGGAGCTCTGGCGTTTCCTGACGCAAAACTTGTCGCTGCCCAATAGCA
CGGCCCAAGCACTCTTGGCCGCCCGTGTGGACCCGCCCGAGGTCTACCACCTGCTCTTTGGTCCCTCATCTGCCCTGGAT
TCACAGTCTGGCCTCCACAAGGGTCAGGAGCCCTGGAGCCGCCTAGGGGGCAATCCCCTGTTCCGGATGGAGGAGCTGCT
GCTGGCTCCTGCCCTCCTGGAGCAGCTCACCTGCACGCCGGGCTCGGGGGAGCTGGGCCGGATCCTCACTGTGCCTGAGA
GTCAGAAGGGAGCCCTGCAGGGCTACCGGGATGCTGTCTGCAGTGGGCAGGCTGCTGCGCGTGCCAGGCGCTTCTCTGGG
CTGTCTGCTGAGCTCCGGAACCAGCTGGACGTGGCCAAGGTCTCCCAGCAGCTGGGCCTGGATGCCCCCAACGGCTCGGA
CTCCTCGCCACAGGCGCCACCCCCACGGAGGCTGCAGGCGCTTCTGGGGGACCTGCTGGATGCCCAGAAGGTTCTGCAGG
ATGTGGATGTCCTGTCGGCCCTGGCCCTGCTACTGCCCCAGGGTGCCTGCACTGGCCGGACCCCCGGACCCCCAGCCAGT
GGTGCGGGTGGGGCGGCCAATGGCACTGGGGCAGGGGCAGTCATGGGCCCCAACGCCACCGCTGAGGAGGGCGCACCCTC
TGCTGCAGCACTGGCCACCCCGGACACGCTGCAGGGCCAGTGCTCAGCCTTCGTACAGCTCTGGGCCGGCCTGCAGCCCA
TCTTGTGTGGCAACAACCGCACCATTGAACCCGAGGCGCTGCGGCGGGGCAACATGAGCTCCCTGGGCTTCACGAGCAAG
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CGACCGCGTCATCCTCAAGGCCAACGAGACTTTTGCTTTTGTGGGCAACGTGACTCACTATGCCCAGGTCTGGCTCAACA
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CTGCGGCTGCACCCCGAGGCACTGAACCTGTCACTGGATGAGCTGCCGCCGGCCCTGAGACAGGACAACTTCTCGCTGCC
CAGTGGCATGGCCCTCCTGCAGCAGCTGGATACCATTGACAACGCGGCCTGCGGCTGGATCCAGTTCATGTCCAAGGTGA
GCGTGGACATCTTCAAGGGCTTCCCCGACGAGGAGAGCATTGTCAACTACACCCTCAACCAGGCCTACCAGGACAACGTC
ACTGTTTTTGCCAGTGTGATCTTCCAGACCCGGAAGGACGGCTCGCTCCCGCCTCACGTGCACTACAAGATCCGCCAGAA
CTCCAGCTTCACCGAGAAAACCAACGAGATCCGCCGCGCCTACTGGCGGCCTGGGCCCAATACTGGCGGCCGCTTCTACT
TCCTCTACGGCTTCGTCTGGATCCAGGACATGATGGAGCGCGCCATCATCGACACTTTTGTGGGGCACGACGTGGTGGAG
CCAGGCAGCTACGTGCAGATGTTCCCCTACCCCTGCTACACACGCGATGACTTCCTGTTTGTCATTGAGCACATGATGCC
GCTGTGCATGGTGATCTCCTGGGTCTACTCCGTGGCCATGACCATCCAGCACATCGTGGCGGAGAAGGAGCACCGGCTCA
AGGAGGTGATGAAGACCATGGGCCTGAACAACGCGGTGCACTGGGTGGCCTGGTTCATCACCGGCTTTGTGCAGCTGTCC
ATCTCCGTGACAGCACTCACCGCCATCCTGAAGTACGGCCAGGTGCTTATGCACAGCCACGTGGTCATCATCTGGCTCTT
CCTGGCAGTCTACGCGGTGGCCACCATCATGTTCTGCTTCCTGGTGTCTGTGCTGTACTCCAAGGCCAAGCTGGCCTCGG
CCTGCGGTGGCATCATCTACTTCCTGAGCTACGTGCCCTACATGTACGTGGCGATCCGAGAGGAGGTGGCGCATGATAAG
ATCACGGCCTTCGAGAAGTGCATCGCGTCCCTCATGTCCACGACGGCCTTTGGTCTGGGCTCTAAGTACTTCGCGCTGTA
TGAGGTGGCCGGCGTGGGCATCCAGTGGCACACCTTCAGCCAGTCCCCGGTGGAGGGGGACGACTTCAACTTGCTCCTGG
CTGTCACCATGCTGATGGTGGACGCCGTGGTCTATGGCATCCTCACGTGGTACATTGAGGCTGTGCACCCAGGCATGTAC
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GGGCAAGACCACCACCATGTCCATCCTGACCGGCCTGTTCCCTCCAACGTCGGGTTCCGCCACCATCTACGGGCACGACA
TCCGCACGGAGATGGATGAGATCCGCAAGAACCTGGGCATGTGCCCGCAGCACAATGTGCTCTTTGACCGGCTCACGGTG
GAGGAACACCTCTGGTTCTACTCACGGCTCAAGAGCATGGCTCAGGAGGAGATCCGCAGAGAGATGGACAAGATGATCGA
GGACCTGGAGCTCTCCAACAAACGGCACTCACTGGTGCAGACATTGTCGGGTGGCATGAAGCGCAAGCTGTCCGTGGCCA
TCGCCTTCGTGGGCGGCTCTCGCGCCATCATCCTGGACGAGCCCACGGCGGGCGTGGACCCCTACGCGCGCCGCGCCATC
TGGGACCTCATCCTGAAGTACAAGCCAGGCCGCACCATCCTTCTGTCCACCCACCACATGGATGAGGCTGACCTGCTTGG
GGACCGCATTGCCATCATCTCCCATGGGAAGCTCAAGTGCTGCGGCTCCCCGCTCTTCCTCAAGGGCACCTATGGCGACG
GGTACCGCCTCACGCTGGTCAAGCGGCCCGCCGAGCCGGGGGGCCCCCAAGAGCCAGGGCTGGCATCCAGCCCCCCAGGT
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CGTCATCTGTGGGCTCTGCCCGTGGCGACGAGGGAGCTGGCTACACCGACGTCTATGGCGACTACCGCCCCCTCTTTGAT
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GCTGGACGGCGGGTGGCTGAAGGTGCGCCAGTTCCACGGGCTGCTGGTCAAACGCTTCCACTGCGCCCGCCGCAACTCCA
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GGTGATCTGCCCCCGCTGGTCCTGTCACCTTCCCAGTACCACAACTACACCCAGCCCCGTGGCAATTTCATCCCCTACGC
CAACGAGGAGCGCCGCGAGTACCGGCTGCGGCTATCGCCCGACGCCAGCCCCCAGCAGCTCGTGAGCACGTTCCGGCTGC
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GAGTCGCGCCTGCTGGCGGCTCGGTTCTTCGACAGCATGTGTCTGGAGTCCTTCACACAGGGGCTGCCACTGTCCAATTT

Figure 6B

CGTGCCACCCCCACCCTCGCCCGCCCCATCTGACTCGCCAGCGTCCCCGGATGAGGACCTGCAGGCCTGGAACGTCTCCC
TGCCGCCCACCGCTGGGCCAGAAATGTGGACGTCGGCACCCTCCCTGCCGCGCCTGGTACGGGAGCCCGTCCGCTGCACC
TGCTCTGCGCAGGGCACCGGCTTCTCCTGCCCCAGCAGTGTGGGCGGGCACCCGCCCCAGATGCGGGTGGTCACAGGCGA
CATCCTGACCGACATCACCGGCCACAATGTCTCTGAGTACCTGCTCTTCACCTCCGACCGCTTCCGACTGCACCGGTATG
GGGCCATCACCTTTGGAAACGTCCTGAAGTCCATCCCAGCCTCATTTGGCACCAGGGCCCCACCCATGGTGCGGAAGATC
GCGGTGCGCAGGGCTGCCCAGGTTTTCTACAACAACAAGGGCTATCACAGCATGCCCACCTACCTCAACAGCCTCAACAA
CGCCATCCTGCGTGCCAACCTGCCCAAGAGCAAGGGCAACCCGGCGGCTTACGGCATCACCGTCACCAACCACCCCATGA
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ATGTCCTTCGTGCCGGCCAGCTTCGTTGTCTTCCTCGTGGCCGAGAAGTCCACCAAGGCCAAGCATCTGCAGTTTGTCAG
CGGCTGCAACCCCATCATCTACTGGCTGGCGAACTACGTGTGGGACATGCTCAACTACCTGGTCCCCGCTACCTGCTGTG
TCATCATCCTGTTTGTGTTCGACCTGCCGGCCTACACGTCGCCCACCAACTTCCCTGCCGTCCTCTCCCTCTTCCTGCTC
TATGGGTGGTCCATCACGCCCATCATGTACCCGGCCTCCTTCTGGTTCGAGGTCCCCAGCTCCGCCTACGTGTTCCTCAT
TGTCATCAATCTCTTCATCGGCATCACCGCCACCGTGGCCACCTTCCTGCTACAGCTCTTCGAGCACGACAAGGACCTGA
AGGTTGTCAACAGTTACCTGAAAAGCTGCTTCCTCATTTTCCCCAACTACAACCTGGGCCACGGGCTCATGGAGATGGCC
TACAACGAGTACATCAACGAGTACTACGCCAAGATTGGCCAGTTTGACAAGATGAAGTCCCCGTTCGAGTGGGACATTGT
CACCCGCGGACTGGTGGCCATGGCGGTTGAGGGCGTCGTGGGCTTCCTCCTGACCATCATGTGCCAGTACAACTTCCTGC
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GGGTACCCAGCCTTCATCTTCCTGGACGAGCCCACCACAGGCATGGACCCCAAGGCCCGGCGCTTCCTCTGGAACCTCAT
CCTCGACCTCATCAAGACAGGGCGTTCAGTGGTGCTGACATCACACAGCATGGAGGAGTGCGAGGCGCTGTGCACGCGGC
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ATCACGGTGCGGACCAAGAGCAGCCAGAGTGTGAAGGACGTGGTGCGGTTCTTCAACCGCAACTTCCCGGAAGCCATGCT
CAAGGAGCGGCACCACACAAAGGTGCAGTACCAGCTCAAGTCGGAGCACATCTCGCTGGCCCAGGTGTTCAGCAAGATGG
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CCTGCTCCGGCCCCGGTCTGCCCCCACGGAGCTCCGGGCACTTGTGGCAGACGAGCCCGAGGACCTGGACACGGAGGACG
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TTTCTCCTGCCCGGCCTCGGAACCAGCTTTTCTCTCTTACGATGAAGGCTGATGCCGAGAGCGGGCTGTGGGCGGAGCTG
GGTCAGTCCCGTATTTATTTTGCTTTGAGAAGAGGCTCCTCTGGCCCTGCTCTCCTGCAGGGAGGTGGCTGTCCCGCGGG
AAGCCATCAGCTTGGGCCAGCTGGCAGGTGGCAGGAATGGAGAAGCTGACCCTGCTGGCCAGGCAAGGGGCCAGACCCCC
CCCAACCCCCAGCTGCCATCGCTCTCCCACCCAGCTTGGCCCCCTGCCCGCCCACCTCCCTGGGAGCCGGGCCTGTACAT
AGCGCACAGATGTTTGTTTTAAATAAATAAACAAAATGTC

Figure 7

h.ABC2 amino acid sequence (SEQ ID NO: 2)

MGFLHQLQLLLWKNVTLKRRSPWVLAFEIFIPLVLFFILLGLRQKKPTISVKEVPFYTAAPLTSAGILPVMQSLCPDGQR
DEFGFLQYANSTVTQLLERLDRVVEEGNLFDPARPSLGSELEALRQHLEALSAGPGTSGSHLDRSTVSSFSLDSVARNPQ
ELWRFLTQNLSLPNSTAQALLAARVDPPEVYHLLFGPSSALDSQSGLHKGQEPWSRLGGNPLFRMEELLLAPALLEQLTC
TPGSGELGRILTVPESQKGALQGYRDAVCSGQAAARARRFSGLSAELRNQLDVAKVSQQLGLDAPNGSDSSPQAPPPRRL
QALLGDLLDAQKVLQDVDVLSALALLLPQGACTGRTPGPPASGAGGAANGTGAGAVMGPNATAEEGAPSAAALATPDTLQ
GQCSAFVQLWAGLQPILCGNNRTIEPEALRRGNMSSLGFTSKEQRNLGLLVHLMTSNPKILYAPAGSEVDRVILKANETF
AFVGNVTHYAQVWLNISAEIRSFLEQGRLQQHLRWLQQYVAELRLHPEALNLSLDELPPALRQDNFSLPSGMALLQQLDT
IDNAACGWIQFMSKVSVDIFKGFPDEESIVNYTLNQAYQDNVTVFASVIFQTRKDGSLPPHVHYKIRQNSSFTEKTNEIR
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AMTIQHIVAEKEHRLKEVMKTMGLNNAVHWVAWFITGFVQLSISVTALTAILKYGQVLMHSHVVIIWLFLAVYAVATIMF
CFLVSVLYSKAKLASACGGIIYFLSYVPYMYVAIREEVAHDKITAFEKCIASLMSTTAFGLGSKYFALYEVAGVGIQWHT
FSQSPVEGDDFNLLLAVTMLMVDAVVYGILTWYIEAVHPGMYGLPRPWYFPLQKSYWLGSGRTEAWEWSWPWARTPRLSV
MEEDQACAMESRRFEETRGMEEEPTHLPLVVCVDKLTKVYKDDKKLALNKLSLNLYENQVVSFLGHNGAGKTTTMSILTG
LFPPTSGSATIYGHDIRTEMDEIRKNLGMCPQHNVLFDRLTVEEHLWFYSRLKSMAQEEIRREMDKMIEDLELSNKRHSL
VQTLSGGMKRKLSVAIAFVGGSRAIILDEPTAGVDPYARRAIWDLILKYKPGRTILLSTHHMDEADLLGDRIAIISHGKL
KCCGSPLFLKGTYGDGYRLTLVKRPAEPGGPQEPGLASSPPGRAPLSSCSELQVSQFIRKHVASCLLVSDTSTELSYILP
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ARCSELTQSQASLQSASSVGSARGDEGAGYTDVYGDYRPLFDNPQDPDNVSLQEVEAEALSRVGQGSRKLDGGWLKVRQF
HGLLVKRFHCARRNSKALFSQILLPAFFVCVAMTVALSVPEIGDLPPLVLSPSQYHNYTQPRGNFIPYANEERREYRLRL
SPDASPQQLVSTFRLPSGVGATCVLKSPANGSLGPTLNLSSGESRLLAARFFDSMCLESFTQGLPLSNFVPPPPSPAPSD
SPASPDEDLQAWNVSLPPTAGPEMWTSAPSLPRLVREPVRCTCSAQGTGFSCPSSVGGHPPQMRVVTGDILTDITGHNVS
EYLLFTSDRFRLHRYGAITFGNVLKSIPASFGTRAPPMVRKIAVRRAAQVFYNNKGYHSMPTYLNSLNNAILRANLPKSK
GNPAAYGITVTNHPMNKTSASLSLDYLLQGTDVVIAIFIIVAMSFVPASFVVFLVAEKSTKAKHLQFVSGCNPIIYWLAN
YVWDMLNYLVPATCCVIILFVFDLPAYTSPTNFPAVLSLFLLYGWSITPIMYPASFWFEVPSSAYVFLIVINLFIGITAT
VATFLLQLFEHDKDLKVVNSYLKSCFLIFPNYNLGHGLMEMAYNEYINEYYAKIGQFDKMKSPFEWDIVTRGLVAMAVEG
VVGFLLTIMCQYNFLRRPQRMPVSTKPVEDDVDVASERQRVLRGDADNDMVKIENLTKVYKSRKIGRILAVDRLCLGVRP
GECFGLLGVNGAGKTSTFKMLTGDESTTGGEAFVNGHSVLKELLQVQQSLGYCPQCDALFDELTAREHLQLYTRLRGISW
KDEARVVKWALEKLELTKYADKPAGTYSGGNKRKLSTAIALIGYPAFIFLDEPTTGMDPKARRFLWNLILDLIKTGRSVV
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LKSEHISLAQVFSKMEQVSGVLGIEDYSVSQTTLDNVFVNFAKKQSDNLEQQETEPPSALQSPLGCLLSLLRPRSAPTEL
RALVADEPEDLDTEDEGLISFEEERAQLSFNTDTLC

OIPE JC11
JUN 24 2002
U.S. PATENT & TRADEMARK OFFICE

UTILITY
Original U.S. or PCT Application

DECLARATION, POWER OF ATTORNEY AND POWER TO INSPECT

As a below named inventor, I hereby declare:

that my residence, post office address and citizenship are as stated below next to my name;

that I verily believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural inventors are named below) of the invention entitled: **NUCLEIC ACID ENCODING HUMAN ABCA TRANSPORTER 2 (ABCA2) AND METHODS OF USE THEREOF**

the specification of which [check one(s) applicable]
__X__ was filed August 31, 2000 as U.S. International Patent Application No. PCT/US00/40789 on which U.S. Patent Application No. 10/088,467, file March 19, 2002 is based.
_____ and was amended by Amendment filed _____ (if applicable); [or];
_____ is attached to this Declaration, Power of Attorney and Power to Inspect;
that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above; and that I acknowledge my duty to disclose information which is material to the examination of this application in accordance wit Rule 56 (a) [37 C.F.R. §1.56(a)].

CLAIM UNDER 35 USC §119(e): I hereby claim the benefit under 35 USC §119(e) of any United States provisional applications listed below:

Provisional Application No.	Filing Date Day/Mo/Year
60/154,839	20 September 1999

POWER OF ATTORNEY: As inventor, I hereby appoint **DANN, DORFMAN, HERRELL AND SKILLMAN, P.C.** of Philadelphia, PA, and the following individual(s) as my attorneys or agents with full power of substitution to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith. **Kathleen D. Rigaut, Ph.D., J.D. Reg. No. 43,047; Patrick J. Hagan, Esq. Req. No. 27,643 and Maria Kourtakis, Esq. Reg. No. 41, 126**

POWER TO INSPECT: I hereby give **DANN, DOREMAN, HERRELL AND SKILLMAN, P.C.** of Philadelphia, PA or its duly accredited representatives power to inspect and obtain copies of the papers on file relating to this application.

SEND CORRESPONDENCE TO: CUSTOMER NUMBER 000110.

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Facsimile: (215) 563-4044

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00

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